A Summary of Current Program 7/1/64
and Preliminary Report of Progress
for 7/1/63 to 6/30/64

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ENTOMOLOGY RESEARCH DIVISION
of the
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
and related work of the
STATE AGRICULTURAL EXPERIMENT STATIONS

Section B

This progress report is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1963, and June 30, 1964. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

UNITED STATES DEPARTMENT OF AGRICULTURE
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AREA NO. 13. BEEF CATTLE, HORSE, AND SWINE INSECTS

Problem. Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious losses. These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed $300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is given to the development of insect sterility, attractants and various

other noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis and equine piroplasmosis.

The Federal scientific effort devoted to research in this area totals 17.8 professional man-years. Of this number, 7.6 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

Research on insects affecting livestock is an important part of the entomological program in the States. Studies on the distribution of pests and their relative importance under the varying conditions in different parts of the country form the basis for more exhaustive research on problem species. Biological information being obtained includes studies of habits, breeding sites, seasonal occurrence and dissemination. Such phenomena as host selection, feeding behavior and host-parasite relationships are investigated by observing and collecting insects from hosts exposed in various habitats. The effects of livestock management practices on insect infestations are being determined by rotating pastures, varying forage types, and stocking rates and shelter manipulation.

Insecticides are being screened to determine their effectiveness as pest toxicants and safety. Systemic insecticides are receiving particular attention. Residue studies are being conducted to obtain basic information on the metabolism of chemicals in animals. Research is underway to determine the interrelationships in the metabolism of combinations of pesticides and to study the physiological and nutritional factors that influence the amount of tissue storage of insecticides.

Insect repellents, attractants and substances interfering with development are being identified and their effectiveness in control evaluated. Information obtained is being considered to determine the feasibility of using such materials in combination with chemosterilants.

A total of 12.6 man years are devoted to this area by the States.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F_3 generation. Field studies on the wild strain of quadrimaculatus in Central Florida showed this species to consistently and predominantly consist of young females (females in the process of laying their first batch of eggs). The only factor that influenced the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58° F and 100% R H for seven days without decreasing their viability. Preliminary attempts to establish laboratory colonies of Aedes infirmatus, Psorophora ferox, and Culex nigripalpus were unsuccessful.

At Corvallis, Oreg., studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Willamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41° to 46° F. When the temperature approached 46° F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days at 75° F, larval and pupal stages required 2-3 days each with little mortality noted at 77° F. The maximum life span of a male and female was 49 to 55 days, respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70° F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences. O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs as 2nd - 4th instar larvae. O. californica was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phreatophytes. Aedes sierrensis require fluctuating water levels for egg hatch. Evaluations of adults from field-collected larvae of 9 species of mosquitoes revealed autogenous egg deposition by only one species - Aedes pullatus.

Studies have confirmed that the major factor responsible for DDT-resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

2. Stable Flies. In Texas studies were conducted in an effort to improve procedures for rearing stable flies and to determine the effects of adverse environment on larval and pupal development. A medium consisting of 30 parts dry C.S.M.A. medium and 100 parts of water (wt/wt) was about optimum for larval survival and development. Lesser and greater amounts of water adversely affected larval survival and development. Second stage larvae survived under a wider range of moisture content than first stage larvae.

In Texas studies were conducted to determine the relative numbers of eggs that mated and unmated female flies would lay by placing individual virgin flies in cages and pairs of males and females in cages. Mated females began laying on the 6th day and laid an average of 112.6 eggs, whereas only 2 of 10 unmated females oviposited beginning on the 17th day, and the average number of eggs was only 5.4. The average life of mated and unmated flies was 25.9 and 19.5 days, respectively. One-day old male stable flies do not mate. The mating percentages for 2- and 3-day old males was 40 and 75, respectively.

At Beltsville, Md., the mating behavior and reproductive capacity of stable flies were studied. Successful matings of flies occurred between one-day-old males and five-day-old females and between one-day-old females and five-day males. The females begin laying eggs when eight days old and lay up to 600 during a lifetime which may be as long as 4-5 weeks.

3. Face Fly. In Nebraska, various combinations of sugar, dried milk, whole milk, egg yolk, citrated blood, dry blood, pollen, and diamalt were tested in an effort to develop an improved diet for colonies of adult face flies. A semiliquid mixture of sugar (6 parts), dry milk (6 parts),egg yolk (1 part) and citrated blood proved most satisfactory, with females producing an average of 26 eggs. Egg production was slightly less when water was substituted for citrated blood.

In Nebraska laboratory studies showed that crowding of face fly larvae in rearing containers reduced the size of the pupae but did not affect the percent of adult emergence. When 100, 200, and 300 larvae were used, the average weights of the pupae were 29, 26, and 21 milligrams, respectively.

In studies of the effect of low temperature, mortality of face fly pupae stored at 40 and 45° F was light to moderate for 3 weeks but was almost complete after 5 weeks. Storage at 36-38° F caused considerable mortality in 1 week and almost complete mortality in 3 weeks. There was very little difference in the survival of 1-, 2-, 3-, and 4-day-old pupae.

Laboratory observations showed that both sexes of the face fly begin mating when about 48 hours old. Adults mated up to 5 times in one day and 10-15 times on successive days. Mating time ranged from 2 minutes to almost 5

hours, averaging 1.0-1.5 hours. Females with sperm in the spermathecae mated an average of 80 minutes, whereas those without sperm mated an average of 42 minutes.

In Nebraska adult face flies were first observed on farms on May 13 but pupae were not found until May 20. Thereafter, adult and pupal numbers increased steadily until late August, then declined rapidly except on one farm. Very few adults or pupae were present by mid-October. There were great differences in the adult and larval populations on different farms but the factors responsible were not determined. Parasitism of pupae averaged only 0.6%.

In Nebraska, ecological studies of cattle droppings in pastures showed a total of 15 species of dipterous larvae. Face fly larval populations were small in May, predominant in June, July, and August, small during September and disappeared by mid-October. Sarcophagid larval breeding (4 species) followed the same trend as the face fly through August but populations were still high in October after face fly larvae disappeared. Over 30 species of Coleoptera were collected over the season, with Aphodius making up over 50% of the total population. Pupal parasitism (Hymenoptera) was relatively light and concentrated in Sarcophagids.

During late September and October, studies in Nebraska showed that released marked adult face flies migrated up to 2 miles within a few days. Marked flies were captured for about 2 weeks after being released. Adult face flies began hibernating on September 13 when the average daily temperature dropped to 56° F. Practically all flies had disappeared from the field by October 11 and an estimated 100,000 flies were observed hibernating on the walls and roof in one barn. Flies were not active even though the temperature was 78° F in the barn. Over 90% of flies collected from the barn in early December and stored at 36-38° F survived but only 4% of those stored under hay in the barn survived the winter. Mortality was at least 99% under normal hibernating conditions where minimum temperatures were as low as -15° F early in January.

Studies at Beltsville, Md., showed that the face fly can complete development from egg to adult in 10 to 12 days (egg stage--1 day, larval stage--4-5 days, pupal stage--5-7 days). Females lay fertile eggs within four days. Eggs are deposited in batches of approximately 20 at 2- to 8-day intervals. Females can lay fertile eggs throughout their life after only a single mating.

4. Horn Fly. In Texas, studies were conducted to compare the suitability of manure from cattle fed alfalfa, sorghum, and prairie hay for the development of horn fly larvae. More larvae matured in the alfalfa manure and the pupae were significantly larger than in the other manures. Sorghum manure was somewhat more satisfactory than prarie hay manure.

5. Screw-worm. Research on the screw-worm continued at Mission, Tex., in support of the sterile-male release program. A line of traps along the

Colorado and Concho Rivers was used to determine the flight range of sterile laboratory reared screw-worm adults marked with P-32 or dyes. In the initial experiment in May and June 1963, over one million flies were released. The most flies were recaptured within 50 miles of the release point, but one was trapped at 65 miles, four at 80 miles, two at 110 miles, two at 140 miles, one at 165 miles, one at 170 miles, and one at 180 miles from the release point. These studies indicated that a barrier sterile fly release zone to keep fertile flies from reaching Texas would need to extend at least 200 miles into Mexico. Additional studies showed that flights in hot weather (June and July) were relatively short and that maximum dispersal occurred in spring and early summer, and again in September and October.

Research was continued to find and develop genetically distinct strains of screw-worm flies. In one study, about 229,000 adults of the Florida strain were examined for inheritable characters. Breeding and backcrossing studies with candidate mutants showed the presence of several strains. Tretamine (a known chemical mutagen) and irradiation were used to induce mutations. In these studies, 23 new strains were produced. Six strains showed unusual larval characters, involving the number of spiracles or the spines on the 11th abdominal segment. All the strains were studied for hardiness and competitiveness and 7 strains discarded when they showed no promise of ever becoming suitable for field use.

Through the SAG test, a technique reported previously for determining mating aggressiveness of sterilized or mutant screw-worm flies, it was determined that one strain of Texas screw-worms contained highly competitive males. Male flies produced in the plant operated by the Animal Disease Eradication Division on beef lung were as aggressive as males from the Entomology Research Division's research colony reared on the standard horse meat diet. Male flies reared in an experimental hydroponic medium were also equivalent in mating ability to flies reared on horse meat.

Studies were continued on the effects of desiccation, starvation, and age of flies at time of release on survival. In studies with a substrain of the original Florida strain of flies selected for resistance to starvation (food and water), adults of the 16th to 19th generations survived as well for 96 hours as the original strain survived for 56 hours. At about 50 hours of starvation, 50% of the unselected strain flies were dead, but only 0.3 to 1.7% of the 19th generation of the new substrain. Sexual aggressiveness of the males in the new substrain appeared normal until the 20th generation when a drop in aggressiveness occurred.

A possible taxonomic difference between laboratory-reared screw-worms and wild screw-worms has been found. Further studies are needed to determine whether this larval characteristic is statistically valid.

In studies of effects of low temperatures, young larvae were less tolerant and 5- to 7-day old pupae were more tolerant than mature larvae. Adults exposed to low temperatures showed greatest tolerance at 1 to 2 hours of

age, as compared with those 1 to 8 days of age. Some abnormal adults were produced when pupae were held at low temperature.

6. House Flies. At Gainesville, Fla., various methods of tagging house flies have been evaluated and developed to study their biology, dispersion, and behavior. An individual male may attempt to copulate with a female at least 6 times in a day. On isolated Grand Turk Island tagged house flies dispersed in all directions from privies where they were released. In some cases flies remained around the privies for 1 or 2 days; in others they dispersed within a day. Using tagged flies it was shown that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the fly population. In another study the ratio of untagged males to untagged females in the buildings was 1.5:1. The total number of flies on the island was somewhere between 200,000 and 300,000. This number of flies represents survivors of the chemosterilant bait applications which are being made on the island.

Eclosion from the pupal stage of house flies exhibted circadian rhythm. A postulate has been made that eclosion begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon, studies on the physiology and biology of house flies were continued. A physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Strains such as this one should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

7. Cattle Grubs. In Texas studies were continued to develop an artificial medium for rearing cattle grub larvae. A medium containing 80% NCTC-109 and 20% agamma calf serum proved most satisfactory for the survival of first stage larvae removed from the gullets of cattle. Survival was best in culture flasks held under near-anerobic conditions and when the medium was changed weekly. Several larvae survived over 110 days.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of horse flies and deer flies. Adults of the spring horse fly, Hybomitra lasiophthalmus, were first observed late in March, reached a peak mid-April, and disappeared by late May. Tabanus vittiger schwardti appeared early in April and by early May small numbers of T. atratus, T. fuscicostatus and several less common species were present. These species were moderately abundant by late May. T. vittiger schwardti was abundant and T. lineola moderately abundant during June and July and T. fuscicostatus light during the same period. Small numbers of six other species were recorded.

In Mississippi an agar medium was developed for the rearing of horse fly larvae. Since the medium is translucent, the movement and development of larvae and their reactions to changes in light and temperature and to vibration are easily observed. Most of the time larvae remain in a vertical position with the terminal spiracle at the air-medium interface. However, even slight vibrations will cause the larvae to penetrate deeper into the medium. Worms or maggots placed in the medium for food are quickly located by the larvae as soon as they penetrate the medium. When approaching the surface for respiration, the larvae move backward and if entrapped air bubbles are encountered, the larvae do not move further until that air supply is exhausted. The larvae tend to seek the darkest portion of the medium when exposed to light and a temperature of 70°-80° F when there is a range of temperatures in the medium.

In Oregon snipe flies (Symphoromyia) are serious pests of animals but efforts to find their breeding areas were unsuccessful for many years. In 1963 heavy larval breeding was found in high altitudes close to water in well-drained situations, under tree trunks and in clumps of blackberry cane. Most of the larvae matured in late June but adults began emerging in mid-June. Adults were very annoying by early July.

9. Lice. In Oregon, louse populations (Bovicola bovis and Linognathus vituli) increased during the summer months on cattle stanchioned in open sunlight. The increases were lower than those noted in previous tests on cattle confined in covered stanchions. When cattle were released to pasture very few lice were evident after 3 weeks. As in earlier studies it was concluded that self-grooming by cattle was largely responsible for the prevention of build-ups of louse populations in the summer months. The ratio of males to females of B. bovis ranged from 1:16 to 1:20, which confirms previous observations that this species can reproduce parthenogenetically.

10. Ticks. In Texas observations were made on the seasonal occurrence of four species of ticks as follows: Lone star tick--Light infestations on cattle in January gradually increasing to heavy infestations throughout April, May, June, and July, declining in August and virtually non-existent by late September. Winter tick--Light infestations on cattle in November gradually increasing to moderate to heavy infestations in December, declining January and February and non-existent by March. Spinose ear tick--Moderately abundant in ears of cattle from November through March, and

abundant from April through October. Tropical horse tick--Surveys in south Texas showed infestations of the tropical horse tick (Dermacentor nitens) on only 3 of 18 ranches. Collections of adults were made and a laboratory colony successfully developed, utilizing horses, rabbits and guinea pigs as hosts. On horses, the life cycle from larvae to engorged adult was 24 days preoviposition period, 3 days; and egg incubation period, 20 days. Females laid an average of about 3500 eggs.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Gainesville, Fla., on the development of insecticides for the control of mosquitoes. These studies included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

Field tests on airplane spray applications of Bayer 41831, Bayer 39007, and malathion as water emulsions or oil solutions for adult mosquito control were conducted. Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 0.1 lb/acre and were more effective than malathion at the same application rate.

Testing of compounds to determine their systemic toxicity against mosquitoes has been continued. Thirty-five compounds previously tested for systemic action in rabbits against body lice, were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

At Corvallis, Oreg., fenthion applied as granular formulations as a mosquito larvicide in log ponds was effective for 7-11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

At Fresno, Calif., in field tests against Culex p. quinquesfasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

2. Stable Flies. At Gainesville, Fla., 141 compounds were evaluated as stable fly larvicides. The most outstanding materials were ethyl dichlorvos, Shell Compound 4072, Bayer 25141, and dichlorvos with LC-50's ranging from 0.21 ppm to 0.64 ppm. Other highly effective materials were phorate, Bayer 24498, Bayer 30237, Bayer 29952, Monsanto CP-7394, Monsanto CP-10613, Bayer 30750, Bayer 39007, Shell SD-8447, Shell SD-8949, Shell SD-8972,

dimethoate, Ciodrin, and Bayer 22492, and Shell SD-8988, all of which had LC-50's ranging from 0.81 ppm to 1.65 ppm.

Six of the promising larvicides were tested against natural populations of stable fly larvae at dosages of 450 and 45 mg/f^2 of surface. At 45 mg/f^2 Bayer 39007 produced 97% to 100% control for 21 days and 94% on the 35th day; Bayer 25141 gave 99% to 100% control for 14 days but only 80% by the 21st day; dimethoate gave poor initial control (71% and 62% after 1 and 7 days) but complete control on the 14th and 21st days; and Ciodrin, dichlorvos, and trichlorfon produced 98% to 99% control within 24 hours after application but were losing effectivenss rapidly by the 7th day. Control after 35 days at both dosage levels was generally low and erratic on all the plots.

Ninety chemicals were evaluated as stable fly adulticides. Forty-eight of the compounds gave at least 80% mortality after 24 hours at a concentration of 0.25%. The most outstanding materials were Ciodrin, Pyrolan, Bayer 39007, Bayer 22684, Shell SD-4092, and Shell SD-3423 with LC-50's ranging from 0.005% to 0.029%. Other outstanding adulticides were Telodrin, Shell SD-3959, Famophos, and Bayer 37341, all of which gave LC-50's ranging from 0.032% to 0.045%.

In Texas, 177 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 4 were Class IV repellents at 5.0% and 7 were Class IV toxicants at 0.25%. The outstanding repellents were ENT Nos. 15029, 27194, 27195, and 27196. The outstanding toxicants were ENT Nos. 17021, 25545, 25841, 25842, 25865, 27122, and 27163.

3. Horn Fly. In field tests in Texas, conventional spray applications of 0.05% of Shell Compound 4072 controlled horn flies for 7 days whereas sprays of 0.1-0.15% were effective for 15-22 days. In Mississippi, Shell Compound 4072 at the same concentrations was effective only about half as long as in Texas, presumably because of heavy rainfall and high humidity. Daily spraying with as little as 0.025% pyrethrins plus 0.125% piperonyl butoxide maintained excellent control of horn flies. Excellent control of horn flies was obtained in hot weather with 5% dusts of carbaryl, ronnel, dioxathion, coumaphos and methoxychlor placed in bags under cattle shelters. In cloudy, cool weather control was poor as cattle did not use the shelters.

In Texas 7 compounds were tested as feed additives for the control of horn fly larval breeding in manure. ENT-25673 at 1 mg/kg and Famophos at 5 mg/kg daily were the only compounds which prevented larval survival. The pathogen, Bacillus thuringiensis, fed at a rate of 4 grams daily inhibited horn fly larval development.

4. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Fifty-two new compounds were screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium. None of the compounds were effective at 0.1 ppm but ENT Nos. 25612, 25780, and 25786 killed all the larvae in 24 hours at 1.0 ppm. In field tests in Mexico, cattle infested with 1-, 2-, 3-, and

4-day old screw-worm larvae were sprayed with 0.05, 0.1, 0.15, 0.2, and 0.25% Shell Compound 4072, or with 0.25% coumaphos at 2 1/2 gallons per animal. One day after treatment, no live larvae were found at 0.2 and 0.25% Shell Compound 4072, but some of the cattle were poisoned. A few larvae survived in cattle sprayed with coumaphos and lower concentrations of Shell Compound 4072. Compound 4072 at 0.1% and higher provided screw-worm control superior to 0.25% coumaphos. In a field test in New Mexico, dusts of 5% coumaphos and ronnel (Korlan) failed to afford 100% control of 1- and 2-day old screw-worm larvae in wounds on cattle, when applied by automatic duster.

5. House Flies. At Gainesville, Fla., tests were conducted with 17 emulsions against natural infestations of house fly larvae in manure at application rates of 100-200 mg/ft^2 of the active ingredient, using 1-2 gallons of liquid spray for each 1,000 square feet of breeding area. Dicapthon caused complete elimination of larvae within 7 days in the only test run with this compound. Indications were that Stauffer N-2404, Bayer 25141, and Shell Compound 4072 also killed all larvae in one test, but Stauffer N-2404 and Bayer 25141 had little, if any, effect in a second test. In one other test at 200 mg and two at 100 mg, Shell Compound 4072 eventually reduced the larval infestation to 13-42% of its original size. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control in all or most of the tests. The remaining compounds were relatively ineffective.

Residual tests were conducted with emulsions of various insecticides against house flies in Florida dairy barns. All were applied at 100 mg/ft^2. Diazinon was included as a standard. The diazinon treatment failed after 1 day in the first test. In the second test, the reduction ranged from 78% to 90% for 5 days, after which the treatment failed. Dimethoate ranged from 83% to 94% for 8 days in one test, and from 91% to 96% for 3 days in the second test, after which the treatments became ineffective. Ciodrin gave reductions from 70% to 90% for 1 week. Fenthion ranged from 80% to 85% control for 5 days in one test, and failed after 1 day in the second. Bayer 39007 exhibited a reduction of 75% the first day, 68% the second, and was ineffective on the 3rd day after application. Endosulfan was ineffective in the first test and failed after 1 day in the second. Shell Compound 4072 was ineffective at 1 day.

At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon and ronnel were superior to the malathion standard against both colonies, but diazinon was indicated to be the better of the two against the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard malathion against the regular colony flies.

At Corvallis, Oreg., research has been continued on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF showed that propyl DEF is

an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion resistant house flies degraded C^{14} malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus resistant house flies and was also effective as a synergist for parathion against resistant flies.

6. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas 64 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Eleven materials showed systemic action in one or both types of administration.

In Texas field tests were conducted on Wyoming cattle to evaluate the systemic effectiveness of six compounds against H. lineatum and H. bovis. ENT-25658 as a 0.5% spray and ENT-26613 in feed at 7.5 mg/kg for 10 days gave 100 and 99% control of both species of grubs, respectively. A single capsule administration of 15 mg/kg of ENT-25832 was 89% effective. The other three materials were relatively ineffective.

In Texas, tests were made on 8 herds of cattle to determine the effectiveness of two applications of 0.25% coumaphos against cattle grubs (H. lineatum). Excellent control (86-100%) was obtained in 4 of 6 herds treated with conventional sprays and in two herds treated by a spray dip machine.

Also in Texas tests were conducted on small numbers of cattle (2-4) with a number of compounds that had shown promise in screening tests and with older effective materials administered in different ways. ENT-25673 at 1 mg/kg, ENT-25684 at 2.5 mg/kg, and Famophos at 5 mg/kg administered for 10 days in feed gave 100% control of cattle grubs and Imidan at 5 mg/kg gave 96% control. Trichlorfon at 1 mg/kg and fenthion at 0.5 mg/kg for 10 days afforded 88% control of grubs. Imidan and trichlorfon gave 99% control when applied at 0.25% in sprays at 1 gallon per animal. As pour-ons, 16.7% Famophos at 100 ml, 2% coumaphos at 250 ml, 2% Imidan at 65 ml, and 2% ENT-25673 at 250 ml/animal gave 100, 99, 98, and 94% control, respectively. Oil pour-ons gave significantly better results than emulsions.

In Oregon extensive tests were run to compare the effectiveness of different volumes and different rates of application of several proven systemics as pour-ons for the control of cattle grubs. The materials tested and amounts that gave effective control of grubs (average over 90%) were as follows:

coumaphos, 4%, 97% control; trichlorphon, 8%, 99% control; fenthion, 1%, 94% control; and Ruelene, 8%, 100% control. In comparative tests as sprays on several herds, one application of coumaphos at 0.5% gave 93-100% control of grubs. Single applications of 0.375% and 0.25% gave 93-98 and 82-91% control, respectively, whereas two sprays of 0.25% a month apart were 93-98% effective.

In Oregon tests were conducted to compare the effectiveness of coumaphos applied as a pour-on and brush-on treatments to different areas of the body. Back brush-on and pour-on treatments were equally effective (94-98% control). Brush-ons on the escutcheon and brisket were moderately effective (81-88% control) but neck and belly applications were relatively ineffective.

7. Horse Flies and Deer Flies. Field tests were conducted to evaluate the effectiveness of 16 materials in protecting cattle from horse flies. One material (ENT-25624) was almost completely effective for 8 hours and provided a high degree of protection for 32 hours but was ineffective after 48 hours. None of the other materials were highly effective after 6-8 hours.

8. Lice. In Mississippi, 9 promising insecticides were evaluated by the "spot-test" method against cattle lice. Five of the insecticides gave 100% control of existing infestations but none showed residual effectiveness beyond 7-9 days. In feeding tests, menazon at 10-15 mg/kg daily for 3 days gave over 95% control of motile lice. Continuous feeding at this rate might eliminate louse infestations.

9. Ticks. In field tests in Texas conventional sprays of 0.1% Shell Compound 4072, 0.75% Dowco 175 and 0.5% toxaphene (standard) were equally effective in controlling the lone star tick. Conventional spray applications of 1 gallon of 0.5% toxaphene were no more effective than 0.38 gallons applied by a spray-dip machine. None of the treatments prevented reinfestations longer than a week or 10 days.

In field tests against the winter tick, toxaphene at 0.5%, Dowco 175 at 0.75%, and Ciodrin at 0.3% gave equally effective immediate control. In comparative tests, applications of 0.5% toxaphene as conventional sprays (1 gal/animal) and by spray-dip machine (0.38 gal/animal) were equally effective.

Field tests against Dermacentor andersoni on cattle were made in Wyoming with recommended sprays (1 gallon per head) of toxaphene (0.5%), coumaphos (0.5%), and dioxathion (0.125%). Toxaphene was also tested alone and in combination with lanolin, Aroclor 5460 (ENT-2589), and other potential extenders at 5%, a dosage which is ten times the recommended toxaphene concentration. However, only 378 ml (instead of 1 gallon) were used and the material sprayed only on the lower neck and brisket area, where the ticks concentrated. With the standard treatments, tick reductions for toxaphene, dioxathion, and coumaphos were 55, 71, and 91%, respectively, after 7 days, and 21, 23, and 57% after 14 days. Toxaphene alone at 5% (378 ml) showed 98, 86, and 20% reductions after 7, 14, and 21 days, respectively. Toxaphene

plus four extenders gave reductions of 98-100, 86-97, and 4-50% after 7, 14, and 21 days, respectively. Results of these tests indicated that toxaphene is more effective than either dioxathion or coumaphos and also that concentrated sprays of toxaphene with additives applied to a limited area on cattle provide greater residual effects than conventional sprays.

In tests in Mexico, sprays of 0.1% and higher of Shell Compound 4072 gave 100% kill of all ticks (Boophilus microplus and Amblyomma cajennense). Sprays of 0.25% coumaphos gave 100% kill of males but were slightly less effective against female ticks. Some molting nymphs survived all treatments and became adults. A few eggs were laid by 1 of 3 females from cattle treated with 0.05% Shell Compound 4072, but none hatched. One of the 19 females from cattle sprayed with 0.25% coumaphos laid a few eggs but larval ticks did not emerge.

In Texas 19 formulations of 16 insecticides were evaluated for control of the spinose ear tick in cattle. All of the materials and formulations gave excellent immediate control of ear ticks. However, after one month the most effective treatments were coumaphos 5% dust (93-96%) followed by 0.25% coumaphos spray (77-81%). The only other treatment showing over 50% control after one month was 0.1% Shell Compound 4072 in a spray.

Small scale field tests were conducted in Texas and Florida to evaluate a number of insecticides for effectiveness against the tropical horse tick (Dermacentor nitens). This species is the vector of equine piroplasmosis in Florida but the disease does not occur in Texas. In Texas 14 formulations of 9 insecticides were tested as ear and body treatments on 60 horses. Treatment affording 99-100% control were as follows: 5% coumaphos dust and 0.25% coumaphos emulsion; 1% dioxathion in mineral oil; 5% ronnel smear; 0.75% lindane in pine oil; 3% lindane smear; 0.1% Shell Compound 4072 emulsion and 1% carbaryl suspension. From 90-99% control was obtained with 0.15% dioxathion emulsion, 0.5% toxaphene emulsion and 0.5% ronnel emulsion.

In Florida a total of 86 horses were treated with 22 formulations of 11 insecticides. Treatments affording highly effective control were as follows: 0.5% toxaphene emulsion, 0.25% coumaphos suspension, 5% coumaphos dust, 0.75% ronnel emulsion, 0.15% dioxathion emulsion and in oil, 0.05% Trithion emulsion and in oil, 0.1% Shell Compound 4072 emulsion and in oil, 3% lindane smear and 0.5% carbaryl suspension and in oil. Trichlorfon fed to one horse at 20 mg/kg/day for 10 days was systemically effective against all stages of D. nitens in the ears of horses. Fenthion at 5 mg/kg also showed promise as a systemic.

C. Insecticide Residue Determinations

1. Residue Studies. In Texas gas chromatographic methods were perfected for the determination of Shell Compound 4072 in animal tissues and milk. Analyses of tissues of animals 7 days after being sprayed with 0.25% of Shell Compound 4072 showed residues of 0.085 ppm only in the omental fat. None

chloroform, and analyzed fluorometrically for coumaphos. The amount of the insecticide deposited on different parts of the body varied from 2.8 to 176.7 micrograms per cm^2 and averaging 51.5 micrograms per cm^2.

2. Toxicity Studies. Research was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

A study to determine the interactions of Vitamin A and phenothiazine drenches with coumaphos was reported for FY 1963. During FY 1964 studies on blood from the animals used in that study included the effects on the Vitamin E of plasma and the Vitamin A and carotene of plasma. There are no significant differences between treatment groups for Vitamin E or for carotene. Vitamin A and carotene values decreased throughout the test in all groups. Plasma Vitamin A was affected by two interactions of treatments. With contaminated coumaphos, animals fed normal diets had lower mean values than those fed additional Vitamin A, whereas those animals treated with normal coumaphos showed no differences in plasma Vitamin A, whether supplemented with A or not.

In animals treated with normal coumaphos the plasma Vitamin A was increased by drenching with phenothiazine/lead arsenate. In those cattle treated with contaminated coumaphos the plasma Vitamin A was lowered by drenching with phenothiazine/lead arsenate.

Atropine, the standard antidote for poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion, the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

A new oxime, TMB-4, has been considerably more effective than previously studied oximes in preventing death and hastening recovery of coumaphos-poisoned cattle. Pralidoxime as 2-PAM chloride (Protopam chloride), in high dosages, this year gave encouraging control of coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Cattle were poisoned by carbaryl then treated with 2-PAM chloride and Promazine. Clinically, the signs of intoxication were markedly increased after

could be detected after 28 days. In a cooperative experiment with the Agricultural Engineering Research Division, two cows were sprayed with Shell Compound 4072. One was sprayed once in the conventional manner with 2 quarts of 0.15% Shell Compound 4072 and the other was sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.10% Shell Compound 4072. Milk samples were collected from both cows for 15 days. Milk from the cows sprayed by the conventional method contained 0.142 ppm of Shell Compound 4072 four hours after spraying, 0.0095 ppm at two days, and less than 0.0003 ppm at eight days after spraying. The level of Shell Compound 4072 residue in the milk from the cows sprayed daily with the automatic mist sprayer ranged from 0.0010 to 0.0003 ppm throughout the observation period.

A method proposed by B. E. Langlois et al (1964. Cleanup of Dairy Products for Analysis of Chlorinated Insecticide Residues by Electroncapture Chromatography, Ag. and Food Chem., 12: 243) has been used with minor modifications for the rapid extraction and clean-up of DDT from milk, followed by gas chromatographic determination. Milk samples were analyzed from two cows, one of which was sprayed once in the conventional manner with 2 quarts of 0.5% DDT and the other sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.05% DDT spray. Milk samples were taken from the combined morning and evening production. The level of DDT in the milk from the cow sprayed daily with the mist sprayer during a 24-day period never exceeded 0.0045 ppm. The level of DDT in the milk from the cow sprayed once in the conventional manner reached a peak of 0.61 ppm 3 days after treatment and fell after 14 days to 0.17 ppm, where it continued through the 21st day.

Two cows were sprayed with ronnel, one in the conventional manner once with 2 quarts of 0.5% ronnel and the other daily with 80-120 ml of an 0.2% mist spray. After 14 days both cows were washed and held for one week, when analysis of the milk showed that both were free of residues. The experiment was repeated on the same cows but the treatments were reversed. Milk samples were collected for 21 days and analyzed. Residues of ronnel were below the limit of sensitivity of the analytical method. The highest levels of residues found in the milk were less than 0.2 ppm 1 or 2 days after application of the 0.5% spray.

Milk samples also were analyzed from two cows using backrubbers treated with 0.5 gallon of a 2% oil solution of ronnel. The cows were forced to walk under the backrubber at least four times a day for 28 days. Milk samples were taken for analysis at intervals during 28 days after use of the rubbers began. There appeared to be no residues in the milk resulting from the use of the backrubbers.

A cooperative experiment with Agricultural Engineering Research Division was set up to study the efficiency of a new automatic spraying device developed for the application of insecticides to livestock. A calf was sprayed twice with 5% coumaphos by passing through the automatic mist spray device. Hair samples were taken from various parts of the animal's body, extracted with

the administration of the two drugs, indicating a potentiating effect of one or both.

Performance standards have been established for emulsions, but not for suspensions. Analyses of dips made with coumaphos, ronnel (Korlan), and Ciodrin were performed. Ronnel performed extremely well, maintaining its concentration precisely during the dipping of 65 sheep in a 600-gallon vat. Ciodrin was a complete failure, the concentration being reduced by more than 60% by the passage of 52 sheep through a 700-gallon vat. Coumaphos showed an essentially uniform tendency to increase in concentration, indicating that sheeps' wool was selectively absorbing more water than toxicant.

The use of present insect chemosterilants for the control of insects must be restricted because of their potential hazards. Although none of these materials are approved for use, studies were continued to determine the hazards to livestock. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa, particularly the deleterious effect upon the tissues that form white blood cells.

Further studies have shown a second effect, teratogensis - that is, the production of monstrosities and defects in the young of animals and birds. Incubating chicken eggs injected with the chemosterilants--apholate, tepa, or metepa at various times showed a disconcerting number of defective chicks. Defects included shortened upper or lower beaks, crossed beaks, absence of legs, curled and fused toes, herniation of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

In further tests with chemosterilants Jersey heifers were selected and divided into groups for treatment and controls. All were observed for 3 months to establish estrous cycles,then the principals were fed apholate daily at a dosage of 1.0 mg/kg. No effect of apholate upon the estrous cycle of the heifers was apparent at the end of 7 months. The heifers were then placed with a Hereford bull for breeding, the apholate feeding continuing at the same dosage. Effects upon implantation of the embryo and upon gestation are currently being observed.

A test was completed with a single survivor of a group of four sheep given 1.0 mg/kg of apholate daily. The test feeding was terminated after the sheep survived 759 daily doses. Principal effect of apholate on this sheep was a reduction of white blood cells and blood platelets. Recovery from these deficiencies has been very slow and is still under study.

Ewes and rams fed a dosage of 0.5 mg/kg of apholate were bred during the feeding period. Ovarian and testicular biopsy tissues did not show evidence of damage by apholate. The ewes lambed normally. White blood cell numbers were slightly reduced. The test was terminated after 494 daily doses had been administered.

A second study was designed to show hematologic and teratogenic (deformity producing) effects that might occur with the feeding of apholate. Rams and ewes were selected, placed on diets containing a dosage of 1.0 mg/kg of apholate and allowed to breed. Three of four test ewes, and both control ewes, delivered normal lambs. One test ewe delivered a deformed lamb.

The deformed lamb showed a lack of eyes and eye nerves, nose, and shortened upper jaw. There was no spleen and the liver was rudimentary in size. A mass outside the body resembled liver. The dam of this lamb had received approximately 189 daily doses of apholate at the time of conception and the lamb was delivered after 345 daily doses had been given.

The response of Brahman cattle to Ciodrin, coumaphos, dioxathion, and Shell Compound 4072 was compared to the responses of cattle of Hereford or other European breeding. Each of the four compounds produced a different result. Coumaphos and Ciodrin produced greater blood cholinesterase depression in Brahman cattle than in cattle of other breeding; Shell Compound 4072 and dioxathion had just the opposite effect.

Research has continued on the treatment of animals poisoned by organic phosphorus compounds. Various oximes were studied for their effectiveness alone or in combination with atropine. Pralidoxime chloride (Protopam chloride) showed good effectivenss alone and in combination with atropine, particularly when the dosage of pralidoxime was kept high and repeated. TMB-4, a relatively new compound, gave good results in the treatment of coumaphos poisoning, the most difficult, usually, to control.

D. Biological Control

1. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microsporidia were collected in June 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1%.

Three of 21 lots of Orthopodomyia californica larvae collected in November 1963 possessed the flagellate, Crithidia fasciculata, which represents the first parasite noted in this remarkably parasite-free mosquito.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge. Identification of the pathogen and its relationship with the host is being undertaken.

A bacterium (Bacillus sphaericus Neide) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory

tests. Limited field trials against mosquito larvae breeding (Culiseta incidens, C. peus and Aedes sierrensis) in rock and tree holes were conducted by treating these with the bacteria. Water temperature ranged from 38° - 48° F. No effect of the bacteria on the larval population was noted. It is possible that the cool temperature prevented build up of the bacteria.

2. Horse Flies and Deer Flies. In Mississippi collections of tabanid larvae in typical habitats showed about 19% of the larvae to be infected with a species of Microsporidia. Healthy larvae readily became infected after feeding on diseased larvae. The organism concentrated in the fatty tissues but also invaded the muscle and salivary glands. Muscles became non-functional when heavily infested.

E. Insect Sterility, Attractants and Other New Approaches to Control

1. Mosquitoes. Studies on chemosterilization of mosquitoes were continued at Gainesville, Fla. Twenty-two compounds were tested as sterilants against larvae of Aedes aegypti. Of these compounds only two caused sterility. In other tests hempa at 50 to 100 ppm gave 99% sterility; ENT-50664 caused complete sterility in the few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants, showed three of these to be highly effective in causing sterility in this species.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Tests with sterile males and normal females of Aedes aegypti indicated that multiple matings of females with successful sperm transfer do not generally occur. However, when the males were sterilized as larvae multiple mating of females did occur, indicating an inability of these sterile males to satisfy the sperm complement necessary in the female spermathecae to inhibit subsequent matings.

Studies with Aedes aegypti mosquitoes and the chemosterilant, apholate, were conducted to determine if resistance to the sterilizing action of this compound could be developed through selection with sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants for this and other species of insects.

In Oregon, ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p.

quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Florida research was continued on factors affecting the attraction of mosquitoes and research initiated on finding specific attractants for mosquitoes. No evidence has been found to show the presence of a chemical sex attractant in Aedes aegypti mosquitoes. Chemotactometer cages were developed to evaluate mosquito response to specific chemicals.

In Oregon, extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

2. Stable Fly. Tests at Beltsville, Md., indicated that stable flies were less attracted to light than either house flies or face flies. Daylight, blacklight BLB, and blacklight BL lamps attracted small percentages of flies in outdoor cages (16% of a test population) whereas, attraction was 43% and 53%, respectively, with house fly and face fly populations.

In Texas topical applications of tretamine at 1.0 µg/fly reduced oviposition by stable flies and no eggs hatched. An application of 4 µg/fly prevented oviposition.

3. Face Fly. In Nebraska a field study was initiated in April and continued to September to determine the effect of releases of sterilized males on natural face fly populations. The test area involved about 1 sq. mile of pasture harboring 25 cattle. The weekly releases of sterile flies averaged 1500 in April, increasing to 20,000 in July, 25,000 in August, and 30,000 in September. The early releases apparently retained the build up of face fly populations but had no control effect during the summer and early fall.

Tests at Beltsville, Md., in outdoor cages indicated that face flies were more attracted by black light than house flies or stable flies. Response of face flies to light occurred principally during periods of evening twilight. In the laboratory red (660 mµ), green (550 mµ), and blacklight (360 mµ) radiation attracted some face flies. A comparison of blacklight and red showed 65-70% of the flies attracted to the blacklight, but only 4% to the red.

4. Horn Fly. In Texas, releases of tepa sterilized male horn flies at a ratio of 3 to 1 to normal males reduced breeding by 60%. The reduction was 16% less than expected, indicating that the sterilized males were not fully competitive. In laboratory feeding tests 5 and 10 ppm of tepa completely prevented oviposition. Feeding of 1.0 ppm reduced oviposition and only 5% of the eggs hatched.

5. Screw-worm. In Texas 22 of 158 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worm when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds

sterilized by both methods of administration. About 18 additional compounds were sufficiently promising in screening to warrant further testing. The sexual vigor and longevity of males sterilized with ENT-50106 or ENT-50450 were reduced but those of males treated with ENT-50716 or ENT-50842 was not affected.

Higher dosages (whether topical or oral) of the chemosterilant, metepa, are required to sterilize screw-worm flies than stable flies. This verifies conclusions drawn from 1962 studies in which screw-worm flies metabolized metepa faster than stable flies and the sterilizing dose was therefore assumed in 1962 to be higher for screw-worms than for stable flies.

When screw-worm cases occur at places more than 100 miles from the known overwintering zone, the question arises about the possibility of sterilized flies recovering from radiation effects. Special tests were therefore made with flies irradiated as 5-, 5 1/2-, and 6 1/2-day-old pupae with 6200 r. Observations of flies maintained for 22 days until 95% had died of old age showed no recovery offertility. Cytological studies of the testes and ovaries of flies treated in this manner up to 31 days old showed a continued degeneration of both testes and ovaries, with no regeneration of germinal tissue. It seems positive, therefore, that the present method of irradiation produces permanently sterilized flies.

Further cytological studies showed the effects of a chemosterilant, tretamine, and gamma irradiation in the screw-worms to be similar, except at the first level of meiosis. Radiation of screw-worm oocytes resulted in many chromosomal aberrations during the 1st and 2nd meiotic divisions of the newly laid eggs; treatment with treatmine, however, resulted in normal-appearing meiosis, followed by visible chromosome damage during cleavage in the embryo larva.

In Texas approximately 90 chemicals and other materials were screened as screw-worm attractants. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. One of the ten materials, ENT-26926X, was highly attractive in some tests, but failed in others. A slightly detectable flowery odor suggested the presence of an impurity, believed to be ethyl isovalerate. Ethyl isovalerate synthesized at Mission and believed to be about 66% pure, was very attractive in several laboratory and field tests. Methyl isovalerate was less attractive. Most of the other 9 promising materials were choline derivatives. Several of these were highly attractive in laboratory and preliminary field tests. An attempt was made to locate pheromones in screw-worms. There was no evidence of a pheromone that would attract males to virgin females, but there was considerable evidence that there may be a pheromone produced by males that is attractive to virgin females.

6. House Fly. Research on the development of sterilization for the control of house flies has been continued. In Florida, screening of new compounds for sterilizing activity, secondary evaluation tests in the laboratory, field experiments, and basic cytological and histological studies have been

continued. Of 338 new compounds screened, 49 sterilized house flies completely at one or more concentrations when fed to adults. Two new compounds, hempa and hemel, which sterilized house flies, were of particular interest because they represent a type of chemical structure not formerly known or shown to cause sterility. In tests with hempa, males were sterilized by feeding for 3 to 5 days on 1% to 2.5% hempa, but not after feeding for only 1 to 2 days. Sterilized males contained motile sperm and transferred motile sperm to the females. Males sterilized with hempa were competitive with normal males in mating with normal females.

Tests were conducted at three poultry farms in Hernando County, Fla., to evaluate the effectiveness of two chemosterilants, metepa and apholate, and an insecticide standard, trichlorfon, for the control of house flies. The breeding site of the house flies was in manure under the poultry cages. Semiweekly bait treatments with 1% of metepa, apholate, or trichlorfon in a sugar-water solution were applied to these droppings. Male and female flies were collected in the poultry houses for sterility determinations. The poultry house treated with metepa bait showed little reduction in the population for the first 2 weeks of treatment. Fly populations progressively decreased for the next 3 weeks, followed by a slight rise the following 2 weeks; at this time, there was such a drastic drop in the population that within 1 week no flies or breeding could be observed. The treatments were discontinued at this time. During the next 2.5 months only three flies were observed and they may have flown in or been carried from some other area. The male sterility was usually above 90% and female sterility was often 100%. Sterility tests were discontinued after the 7th week because of the complete lack of adults in the test area. The fly population at the poultry house treated with apholate remained at a fairly constant level for the first 6 weeks of testing, after which a decrease was observed for 2 weeks, but the population increased thereafter and remained at the early post-treatment levels until the conclusion of the test. Sterility induced in both sexes was rapid and nearly complete. The fly densities at the poultry house treated with trichlorfon decreased immediately after treatment and remained at about the same level throughout the test period.

Experiments have been continued to determine whether a dosage of a chemosterilant too low to prevent complete hatching or adult emergence in the flies to which it was administered might, by the accumulation of genetic injuries, eventually reduce or eliminate reproduction after successive generations of exposure. The experimental colony in which successive generations were fed 0.01% of apholate in the adult food, is now in the F_{40} generation. The number of progeny from treated flies pupating in the first five generations was not substantially different from the standard, but then began to decline. Only 20% to 40% of the population were reaching the pupal stage by the 34th to 39th generations. When this colony was in the F_{22} generation, two groups of 100 pupae each were taken from the colony and two new colonies started. These were reared on regular fly food for 6 generations. The rate of pupation still varied from about 7% to 45%.

Tests were conducted to determine the effects of metepa on the chromosomes of adult male house flies. Broken chromosomes in the metephase stage were observed in males that had been allowed to feed for 4 days on food treated with 0.5% metepa. In some males that had fed for 4 days on treated food the chromosomes in the prophase stage appeared unbroken but they stained atypically. To determine whether gamma irradiation also produced this effect, house flies in the late pupal stage were exposed to a sterilizing dosage of 2850 r from a cobalt-60 source. Twenty-four to twenty-five hours after irradiation, when the adult males were 15 to 16 hours old, the testes were removed and squash preparations were made. Chromosomal damage obtained by irradiation was strikingly similar to that produced by metepa. Fragmentation ranged from mild to severe, with atypical staining of the nuclear material.

In large outdoor cage tests at Beltsville, Md., usually 40-64%, but never over 75%, of the house flies present in the cage were attracted to the radiant energy emitted by daylight, blacklight BLB, and blacklight BL fluorescent lamps. Most of the flies which responded were attracted during the first night of exposure.

F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Mississippi and Texas in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State experimental stations to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. In Mississippi, tests involved the exposure of three groups of cattle to insect attack-one group continuously, one group at night (1 hour after sunset to 1 hour before sunrise), and one group in daytime (1 hour after sunrise to 1 hour before sunset). Each group was exposed daily with animals infected with anaplasmosis from June 5 to July 10. All of the continuously exposed animals developed the disease within 4-5 weeks. At least one and possibly two of the other groups also contracted the disease. However, the tests were considered inconclusive and will be repeated in an effort to obtain more precise information on the relative importance of night and day feeding insects in anaplasmosis transmission. During the period in which transmission presumably occurred in these tests the principal daytime biting species were the horse flies, T. vittiger schwardti, T. lineola and T. fuscicostatus, and the mosquito, P. confinnis. No horse flies were active at night so biting was confined to mosquitoes, principally P. confinnis and A. quadrimaculatus.

During the summer animal-baited and light traps were operated at night in the Mississippi Delta in an effort to correlate bovine anaplasmosis transmission in an area as a whole with the relative abundance and feeding activity of mosquitoes and horse flies by species. The predominant species of mosquitoes based on animal traps early in the summer were Aedes vexans (about 50%), Aedes sticticus (about 20%), Anopheles quadrimaculatus (about 10%), Culex erraticus (about 6%), and Psorophora confinnis (about 4%). Later in the summer the predominant species were A. quadrimaculatus (36%),

C. erraticus (31%), Psorophora confinnis (21%), and A. vexans (8%). Mosquitoes were greatest on a tethered steer, considerably less on a steer in a trap, and least in a light trap. Over 5000 mosquitoes were estimated to have fed on the tethered steer during one 12-hour test period. The feeding of this number undoubtedly adversely affected the health and comfort of the animal.

In Texas monthly surveys were continued to determine the identity of external parasites on infected (anaplasmosis) and clean herds of cattle. Light infestations of the lone star tick were noted in January, heavy infestations from April through July, and very few after early September. The winter tick appeared in November and was abundant during December and January, and almost nonexistent by March. The spinose ear tick was moderately abundant to abundant throughout the year. Horn flies appeared in April and were present until November. Cattle were sprayed periodically to keep the adult population at low to moderate levels. Light cattle louse infestations were noted from October to April. As a result of prompt segregation of reactor cattle, an anaplasmosis-free herd of 75 cattle has been developed during the past several years.

At Beltsville, Md., transmission experiments and cytological studies with experimental vectors were continued in cooperation with the Animal Disease and Parasite Research Division. Colonization of the Pacific coast tick (Dermacentor occidentalis) has been accomplished and one hereditary transmission experiment has been conducted. The adults were allowed to feed on a calf during the clinical stages of anaplasmosis. The progeny of these adults were then tested on a susceptible splenectomized calf. Anaplasmosis was not transmitted; however, proof of susceptibility by challenge has not yet been completed. A single engorged Dermacentor andersoni female was received from the Department of Veterinary Science, University of Nevada. This specimen was taken from a deer that, on subinoculation of blood with a splenectomized calf, was infected with A. marginale. The larval progeny of this female were placed on a splenectomized calf for testing, but the larvae did not attach and feed and all the ticks were lost.

Cytological studies on vector species have been continued at Beltsville. Infected and non-infected D. andersoni and D. occidentalis have been prepared for electron microscopy, but tissue examinations have not been completed.

2. Equine piroplasmosis. At Beltsville, Md., investigations on the vectors and experimental transmission of equine piroplasmosis were initiated in cooperation with the Animal Disease and Parasite Research Division. One experimental facility was completed in April of 1964 and transmission tests with Rhipicephalus sanguineus, Dermacentor andersoni, and Dermacentor occidentalis, were initiated. In all tests, the ticks were fed on horses that were past the acute stage of piroplasmosis infection and were considered to be carriers of Babesia caballi. The test with R. sanguineus was a stage-to-stage transmission trial with the ticks being fed on the infected horse as nymphs, and tested on a susceptible horse as adults. There was no

transmission. The tests with D. andersoni and D. occidentalis were hereditary transmission trials. Adult ticks were fed on the carrier horses and the larval progeny of these ticks were allowed to feed on susceptible horses. There was no transmission in either of these tests, however, most of the larval ticks did not feed to repeltion. Two tests with the progeny of field-collected Dermacentor nitens were also negative.

Cytological studies on the ticks that fed on the infected horses and on D. andersoni eggs from the females that fed on the infected horse were initiated. Immuno fluorescence studies on smears of salivary glands, reproductive organs, and gut tissue did not reveal developmental forms of Babesia caballi.

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AREA NO. 14. DAIRY CATTLE INSECTS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed $200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, non-residue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored further to determine their feasibility for the control of several dairy-cattle pests. Research should be continued to support the Southwestern screw-worm eradication campaign. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating and

controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif.

The Federal scientific effort devoted to research in this area totals 16.6 professional man-years. Of this number 6.4 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

Valuable information on insects affecting dairy cattle is being provided by research in the States. Studies are in progress to determine the abundance, geographical distribution, seasonal variations and economic importance of pest species. Rearing methods are being developed to provide insect specimens (1) for laboratory studies involving the effects of ecological factors on growth and survival; (2) for studying the micro-organisms normally present in pest insects; (3) for cattle disease transmission tests performed to determine which insects may serve as vectors; and (4) for control studies.

Various substances are being evaluated for their attractant or repellent effects on such pest insects as flies. Those attractants which exert a significant effect are incorporated as baits with new insecticides or chemosterilants. Various other application methods are also being evaluated.

The development of resistance to insecticides in flies has brought about research to determine methods of combatting it as well as initiating a search for new chemicals. Studies are in progress to determine the effects of repeated heavy insecticide dosages as opposed to light doses, and the influence of fly behavior, development and reproductive capacity on resistance. The mechanism of resistance in the insect in relation to penetration of the integument, distribution, activation, degradation and excretion of the insecticides is also being investigated.

Biological control research is being performed to determine the value of natural agents as supplementary control measures. On dairy cattle, materials and techniques of application are being tested for their effects on weight gain, milk production, milk contamination and animal health as well as pest control. Milk and tissues are being recovered from treated animals and examined for pesticide content. Detection of metabolites as well as the original compounds is being emphasized.

There are 6.7 man-years devoted by the States to research on insects affecting dairy cattle.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F_3 generation. Field studies on the wild strain of quadrimaculatus in Central Florida showed this species to consistently and predominantly consist of young females (females in the process of laying their first batch of eggs). The only factor that influenced the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58°F. and 100% R.H. for seven days without decreasing their viability. Preliminary attempts to establish laboratory colonies of Aedes infirmatus, Psorophora ferox, and Culex nigripalpus were unsuccessful.

At Corvallis, Oreg. studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Williamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41°to 46°F. When the temperature approached 46°F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days at 75°F, larval and pupal stages required 2-3 days each with little mortality noted at 77°F. The maximum life span of a male and female was 49 and 55 days, respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70°F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences. O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs as 2nd - 4th instar larvae. O. californica

was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phreatophytes. Aedes sierrensis require fluctuating water levels for egg hatch. Evaluations of adults from field-collected larvae of 9 species of mosquitoes revealed autogenous egg deposition by only one species - Aedes pullatus.

Studies have confirmed that the major factor responsible for DDT-resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

2. Stable flies. In Texas studies were conducted in an effort to improve procedures for rearing stable flies and to determine the effects of adverse environment on larval and pupal development. A medium consisting of 30 parts dry C.S.M.A. medium and 100 parts of water (wt/wt) was about optimum for larval survival and development. Lesser and greater amounts of water adversely affected larval survival and development. Second stage larvae survived under a wider range of moisture content than first stage larvae.

In Texas studies were conducted to determine the relative numbers of eggs that mated and unmated female flies would lay by placing individual virgin flies in cages and pairs of males and females in cages. Mated females began laying on the 6th day and laid an average of 112.6 eggs, whereas only 2 of 10 unmated females oviposited beginning on the 17th day, and the average number of eggs was only 5.4. The average life of mated and unmated flies was 25.9 and 19.5 days, respectively. One-day old male stable flies do not mate. The mating percentages for 2- and 3-day old males was 40 and 75, respectively.

At Beltsville, Md., the mating behavior and reproductive capacity of stable flies were studied. Successful matings of flies occurred between one-day-old males and five-day-old females and between one-day-old females and five-day-old males. The females begin laying eggs when eight days old and lay up to 600 during a lifetime which may be as long as 4-5 weeks.

3. Face fly. In Nebraska, various combinations of sugar, dried milk, whole milk, egg yolk, citrated blood, dry blood, pollen, and diamalt were tested in an effort to develop an improved diet for colonies of adult face flies. A semiliquid mixture of sugar (6 parts), dry milk (6 parts), egg yolk (1 part) and citrated blood proved most satisfactory, with females producing an average of 26 eggs. Egg production was slightly less when water was substituted for citrated blood.

In Nebraska laboratory studies showed that crowding of face fly larvae in rearing containers reduced the size of the pupae but did not affect the percent of adult emergence. When 100, 200, and 300 larvae were used, the average weights of the pupae were 29, 26, and 21 milligrams, respectively.

In studies of the effect of low temperature, mortality of face fly pupae stored at 40 and 45°F was light to moderate for 3 weeks but was almost

complete after 5 weeks. Storage at 36-38°F caused considerable mortality in 1 week and almost complete mortality in 3 weeks. There was very little difference in the survival of 1, 2, 3, and 4 day-old pupae.

Laboratory observations showed that both sexes of the face fly begin mating when about 48 hours old. Adults mated up to 5 times in one day and 10-15 times on successive days. Mating time ranged from 2 minutes to almost 5 hours, averaging 1.0-1.5 hours. Females with sperm in the spermathecae mated an average of 80 minutes, whereas those without sperm mated an average of 42 minutes.

In Nebraska adult face flies were first observed on farms on May 13, but pupae were not found until May 20. Thereafter, adult and pupal numbers increased steadily until late August, then declined rapidly except on one farm. Very few adults or pupae were present by mid-October. There were great differences in the adult and larval populations on different farms but the factors responsible were not determined. Parasitism of pupae averaged only 0.6%.

In Nebraska ecological studies of cattle droppings in pastures showed a total of 15 species of dipterous larvae. Face fly larval populations were small in May, predominant in June, July, and August, small during September and disappeared by mid-October. Sarcophagid larval breeding (4 species) followed the same trend as the face fly through August but populations were still high in October after face fly larvae disappeared. Over 30 species of Coleoptera were collected over the season, with Aphodius making up over 50% of the total population. Pupal parasitism (Hymenoptera) was relatively light and concentrated in Sarcophagids.

During late September and October, studies in Nebraska showed that released marked adult face flies migrated up to 2 miles within a few days. Marked flies were captured for about 2 weeks after being released. Adult face flies began hibernating on September 13 when the average daily temperature dropped to 56°F. Practically all flies had disappeared from the field by October 11 and an estimated 100,000 flies were observed hibernating on the walls and roof in one barn. Flies were not active even though the temperature was 78°F in the barn. Over 90% of flies collected from the barn in early December and stored at 36-38°F survived but only 4% of those stored under hay in the barn survived the winter. Mortality was at least 99% under normal hibernating conditions where minimum temperatures were as low as -15°F early in January.

Studies at Beltsville, Md., showed that the face fly can complete development from egg to adult in 10 to 12 days (egg stage -- 1 day, larval stage -- 4-5 days, pupal stage -- 5-7 days). Females lay fertile eggs within four days. Eggs are deposited in batches of approximately 20 at 2- to 8-day intervals. Females can lay fertile eggs throughout their life after only a single mating.

4. Horn Fly. In Texas, studies were conducted to compare the suitability of manure from cattle fed alfalfa, sorghum, and prairie hay for the development of horn fly larvae. More larvae matured in the alfalfa manure and the pupae were significantly larger than in the other manures. Sorghum manure was somewhat more satisfactory than prarie hay manure.

5. Screw-worm. Research on the screw-worm continued at Mission, Tex., in support of the sterile male release program. A line of traps along the Colorado and Concho Rivers was used to determine the flight range of sterile, laboratory reared screw-worm adults marked with P-32 or dyes. In the initial experiment in May and June 1963, over one million flies were released. The most flies were recaptured within 50 miles of the release point, but one was trapped at 65 miles, four at 80 miles, two at 110 miles, two at 140 miles, one at 165 miles, one at 170 miles, and one at 180 miles from the release point. These studies indicated that a barrier (sterile fly release zone) to keep fertile flies from reaching Texas would need to extend at least 200 miles into Mexico. Additional studies showed that flights in hot weather (June and July) were relatively short and that maximum dispersal occurred in spring and early summer, and again in September and October.

Research was continued to find and develop genetically distinct strains of screw-worm flies. In one study, about 229,000 adults of the Florida strain were examined for inheritable characters. Breeding and backcrossing studies with candidate mutants showed the presence of several strains. Tretamine (a known chemical mutagen) and irradiation were used to induce mutations. In these studies, 23 new strains were produced. Six strains showed unusual larval characters, involving the number of spiracles or the spines on the 11th abdominal segment. All the strains were studied for hardiness and competitiveness and 7 strains discarded when they showed no promise of ever becoming suitable for field use.

Through the SAG test, a technique reported previously for determining mating aggressiveness of sterilized or mutant screw-worm flies, it was determined that one strain of Texas screw-worms contained highly competitive males. Male flies produced in the plant operated by the Animal Disease Eradication Division on beef lung were as aggressive as males from the Entomology Research Division's research colony reared on the standard horse meat diet. Male flies reared in an experimental hydroponic medium were also equivalent in mating ability to flies reared on horse meat.

Studies were continued on the effects of desiccation, starvation, and age of flies at time of release on survival. In studies with a substrain of the original Florida strain of flies selected for resistance to starvation (food and water), adults of the 16th to 19th generations survived as well for 96 hours as the original strain survived for 56 hours. At about 50 hours of starvation, 50% of the unselected strain flies were dead, but only 0.3 to 1.7% of the 19th generation of the new substrain. Sexual aggressiveness of the males in the new substrain appeared normal until the 20th generation when a drop in aggressiveness occurred.

A possible taxonomic difference between laboratory-reared screw-worms and wild screw-worms has been found. Further studies are needed to determine whether this larval characteristic is statistically valid.

In studies of effects of low temperatures, young larvae were less tolerant and 5- to 7-day old pupae were more tolerant than mature larvae. Adults exposed to low temperatures showed greatest tolerance at 1 to 2 hours of age, as compared with those 1 to 8 days of age. Some abnormal adults were produced when pupae were held at low temperature.

6. House Flies. At Gainesville, Fla., various methods of tagging house flies have been evaluated and developed to study their biology, dispersion, and behavior. An individual male may attempt to copulate with a female at least 6 times in a day. On isolated Grand Turk Island tagged house flies dispersed in all directions from privies where they were released. In some cases flies remained around the privies for 1 or 2 days; in others they dispersed within a day. Using tagged flies it was shown that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the fly population. In another study the ratio of untagged males to untagged females in the buildings was 1.5:1. The total number of flies on the island was somewhere between 200,000 and 300,000. This number of flies represents survivors of the chemosterilant bait applications which are being made on the island.

Eclosion from the pupal stage of house flies exhibited circadian rhythm. A postulate has been made that eclosion begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon, studies on the physiology and biology of house flies were continued. A physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Strains such as this one should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and

Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

7. Cattle Grubs. In Texas studies were continued to develop an artificial medium for rearing cattle grub larvae. A medium containing 80% NCTC-109 and 20% agamma calf serum proved most satisfactory for the survival of first stage larvae removed from the gullets of cattle. Survival was best in culture flasks held under near-anerobic conditions and when the medium was changed weekly. Several larvae survived over 110 days.

8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of horse flies and deer flies. Adults of the spring horse fly, Hybomitra lasiophthalmus, were first observed late in March, reached a peak in mid-April and disappeared by late May. Tabanus vittiger schwardti appeared early in April and by early May small numbers of T. atratus, T. fuscicostatus and several less common species were present. These species were moderately abundant by late May. T. vittiger schwardi was abundant and T. lineola moderately abundant during June and July and T. fuscicostatus light during the same period. Small numbers of six other species were recorded.

In Mississippi an agar medium was developed for the rearing of horse fly larvae. Since the medium is translucent, the movement and development of larvae and their reactions to changes in light and temperature and to vibration are easily observed. Most of the time larvae remain in a vertical position with the terminal spiracle at the air-medium interface. However, even slight vibrations will cause the larvae to penetrate deeper into the medium. Worms or maggots placed in the medium for food are quickly located by the larvae as soon as they penetrate the medium. When approaching the surface for respiration, the larvae move backward and if entrapped air bubbles are encountered, the larvae do not move further until that air supply is exhausted. The larvae tend to seek the darkest portion of the medium when exposed to light and a temperature of 70^{o}-80^{o}F when there is a range of temperatures in the medium.

In Oregon snipe flies (Symphoromyia) are serious pests of animals but efforts to find their breeding areas were unsuccessful for many years. In 1963 heavy larval breeding was found in high altitudes close to water in well-drained situations, under tree trunks and in clumps of blackberry cane. Most of the larvae matured in late June but adults began emerging in mid-June. Adults were very annoying by early July.

9. Lice. In Oregon louse populations (Bovicola bovis and Linognathus vituli) increased during the summer months on cattle stanchioned in open sunlight. The increases were lower than those noted in previous tests on cattle confined in covered stanchions. When cattle were released to pasture very few lice were evident after 3 weeks. As in earlier studies it was concluded that self-grooming by cattle was largely responsible for the prevention of build-ups of louse populations in the summer months. The ratio of

males to females of B. bovis ranged from 1:16-1:20, which confirms previous observations that this species can reproduce parthenogenetically.

10. Ticks. In Texas observations were made on the seasonal occurrence of four species of ticks as follows: Lone star tick--Light infestations on cattle in January gradually increasing to heavy infestations throughout April, May, June, and July, declining in August and virtually non-existent by late September. Winter tick--Light infestations on cattle in November gradually increasing to moderate to heavy infestations in December, declining during January and February and non-existent by March. Spinose ear tick--Moderately abundant in ears of cattle from November through March, and abundant from April through October.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Gainesville, Fla., on the development of insecticides for the control of mosquitoes. These studies included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

Field tests on airplane spray applications of Bayer 41831, Bayer 39007, and malathion as water emulsions or oil solutions for adult mosquito control were conducted. Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 0.1 lb/acre and were more effective than malathion at the same application rate.

Testing of compounds to determine their systemic toxicity against mosquitoes has been continued. Thirty-five compounds previously tested for systemic action in rabbits against body lice, were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

At Corvallis, Oreg., fenthion applied as granular formulations as a mosquito larvicide in log ponds was effective for 7-11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

At Fresno, Calif., in field tests against Culex p. quinquefasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

2. Stable flies. At Gainesville, Fla., 141 compounds were evaluated as stable fly larvicides. The most outstanding materials were ethyl dichlorvos,

Shell Compound 4072, Bayer 25141, and dichlorvos with LC-50's ranging from 0.21 ppm to 0.64 ppm. Other highly effective materials were phorate, Bayer 24498, Bayer 30237, Bayer 29952, Monsanto CP-7394, Monsanto CP-10613, Bayer 30750, Bayer 39007, Shell SD-8447, Shell SD-8949, Shell SD-8972, dimethoate, Ciodrin, and Bayer 22492, and Shell SD-8988, all of which had LC-50's ranging from 0.81 ppm to 1.65 ppm.

Six of the promising larvicides were tested against natural populations of stable fly larvae at dosages of 450 and 45 mg/ft^2 of surface. At 45 mg/ft^2 Bayer 39007 produced 97% to 100% control for 21 days and 94% on the 35th day; Bayer 25141 gave 99% to 100% control for 14 days but only 80% by the 21st day; dimethoate gave poor initial control (71% and 62% after 1 and 7 days) but complete control on the 14th and 21st days; and Ciodrin, dichlorvos, and trichlorfon produced 98% to 99% control within 24 hours after application but were losing effectiveness rapidly by the 7th day. Control after 35 days at both dosage levels was generally low and erratic on all the plots.

Ninety chemicals were evaluated as stable fly adulticides. Forty-eight of the compounds gave at least 80% mortality after 24 hours at a concentration of 0.25%. The most outstanding materials were Ciodrin, Pyrolan, Bayer 39007, Bayer 22684, Shell SD-4092, and Shell SD-3423 with LC-50's ranging from 0.005% to 0.029%. Other outstanding adulticides were Telodrin, Shell SD-3959, Famophos, and Bayer 37341, all of which gave LC-50's ranging from 0.032% to 0.045%.

In Texas, 177 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 4 were class IV repellents at 5.0% and 7 were Class IV toxicants at 0.25%. The outstanding repellents were ENT Nos. 15029, 27194, 27195, and 27196. The outstanding toxicants were ENT Nos. 17021, 25545, 25841, 25842, 25865, 27122, and 27163.

3. Horn Fly. In field tests in Texas, conventional spray applications of 0.05% of Shell Compound 4072 controlled horn flies for 7 days whereas sprays of 0.1-0.15% were effective for 15-22 days. In Mississippi, Shell Compound 4072 at the same concentrations was effective only about half as long as in Texas, presumably because of heavy rainfall and high humidity. Daily spraying with as little as 0.025% pyrethrins plus 0.125% piperonyl butoxide maintained excellent control of horn flies. Excellent control of horn flies was obtained in hot weather with 5% dusts of carbaryl, ronnel, dioxathion, coumaphos and methoxychlor placed in bags under cattle shelters. In cloudy, cool weather control was poor as cattle did not use the shelters.

In Texas, 7 compounds were tested as feed additives for the control of horn fly larval breeding in manure. ENT-25673 at 1 mg/kg and Famophos at 5 mg/kg daily were the only compounds which prevented larval survival. The pathogen, Bacillus thuringiensis, fed at a rate of 4 grams daily, inhibited horn fly larval development.

4. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Fifty-two new compounds were screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium. None of the compounds were effective at 0. 1 ppm but ENT Nos. 25612, 25780, and 25786 killed all the larvae in 24 hours at 1.0 ppm. In field tests in Mexico, cattle infested with 1-, 2-, 3-, and 4-day old screw-worm larvae were sprayed with 0.05, 0.1, 0.15, 0.2, and 0.25% Shell Compound 4072, or with 0.25% coumaphos at 2 1/2 gallons per animal. One day after treatment, no live larvae were found at 0.2 and 0.25% Shell Compound 4072, but some of the cattle were poisoned. A few larvae survived in cattle sprayed with coumaphos and lower concentrations of Shell Compound 4072 . Compound 4072 at 0.1% and higher provided screw-worm control superior to 0.25% coumaphos. In a field test in New Mexico, dusts of 5% coumaphos and ronnel (Korlan) failed to afford 100% control of 1- and 2-day old screw-worm larvae in wounds on cattle, when applied by automatic duster.

5. House Flies. At Gainesville, Fla., tests were conducted with 17 emulsions against natural infestations of house fly larvae in manure at application rates of 100-200 mg/ft^2 of the active ingredient, using 1-2 gallons of liquid spray for each 1,000 square feet of breeding area. Dicapthon caused complete elimination of larvae within 7 days in the only test run with this compound. Indications were that Stauffer N-2404, Bayer 25141, and Shell Compound 4072 also killed all larvae in one test, but Stauffer N-2404 and Bayer 25141 had little, if any, effect in a second test. In one other test at 200 mg and two at 100 mg., Shell Compound 4072 eventually reduced the larval infestation to 13%-42% of its original size. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control in all or most of the tests. The remaining compounds were relatively ineffective.

Residual tests were conducted with emulsions of various insecticides against house flies in Florida dairy barns. All were applied at 100 mg/ft^2. Diazinon was included as a standard. The diazinon treatment failed after 1 day in the first test. In the second test, the reduction ranged from 78% to 90% for 5 days, after which the treatment failed. Dimethoate ranged from 83% to 94% for 8 days in one test, and from 91% to 96% for 3 days in the second test, after which the treatments became ineffective. Ciodrin gave reductions from 70% to 90% for 1 week. Fenthion ranged from 80% to 85% control for 5 days in one test, and failed after 1 day in the second. Bayer 39007 exhibited a reduction of 75% the first day, 68% the second, and was ineffective on the 3rd day after application. Endosulfan was ineffective in the first test and failed after 1 day in the second. Shell Compound 4072 was ineffective at 1 day.

At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon and ronnel were superior to the malathion standard against both colonies, but diazinon was indicated to be the better of the two against

the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard malathion against the regular colony flies.

At Corvallis, Oreg., research has been continued on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF showed that propyl DEF is an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion resistant house flies degraded C^{14} malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus resistant house flies and was also effective as a synergist for parathion against resistant flies.

6. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas, 64 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Eleven materials showed systemic action in one or both types of administration.

In Texas, field tests were conducted on Wyoming cattle to evaluate the systemic effectiveness of six compounds against H. lineatum and H. bovis. ENT-25658 as a 0.5% spray and ENT-26613 in feed at 7.5 mg/kg for 10 days gave 100 and 99% control of both species of grubs, respectively. A single capsule administration of 15 mg/kg of ENT-25832 was 89% effective. The other three materials were relatively ineffective.

In Texas, tests were made on 8 herds of cattle to determine the effectiveness of two applications of 0.25% coumaphos against cattle grubs (H. lineatum). Excellent control (86-100%) was obtained in 4 of 6 herds treated with conventional sprays and in two herds treated by a spray dip machine.

Also in Texas tests were conducted on small numbers of cattle (2-4) with a number of compounds that had shown promise in screening tests and with older effective materials administered in different ways. ENT-25673 at 1 mg/kg; ENT-25684 at 2.5 mg/kg, and Famophos at 5 mg/kg administered for 10 days in feed gave 100% control of cattle grubs and Imidan at 5 mg/kg gave 96% control. Trichlorfon at 1 mg/kg and fenthion at 0.5 mg/kg for 10 days afforded 88% control of grubs. Imidan and trichlorfon gave 99% control when applied at 0.25% in sprays at 1 gallon per animal. As pour-ons, 16.7% Famophos at 100 ml, 2% coumaphos at 250 ml, 2% Imidan at 65 ml and 2%

ENT-25673 at 250 ml/animal gave 100, 99, 98, and 94% control, respectively. Oil pour-ons gave significantly better results than emulsions.

In Oregon, extensive tests were run to compare the effectiveness of different volumes and different rates of application of several proven systemics as pour-ons for the control of cattle grubs. The materials tested and the amounts that gave effective control of grubs (average over 90%) were as follows: coumaphos, 4%, 97% control; trichlorophon 8%, 99% control; fenthion 1%, 94% control; and Ruelene, 8%, 100% control. In comparative tests as sprays on several herds, one application of coumaphos at 0.5% gave 93-100% control of grubs. Single applications of 0.375% and 0.25% gave 93-98 and 82-91% control, respectively, whereas two sprays of 0.25% a month apart were 93-98% effective.

In Oregon, tests were conducted to compare the effectiveness of coumaphos applied as pour-on and brush-on treatments to different areas of the body. Back brush-on and pour-on treatments were equally effective (94-98% control). Brush-ons on the escutcheon and brisket were moderately effective (81-88% control) but neck and belly applications were relatively ineffective.

7. Horse Flies and Deer Flies. Field tests were conducted to evaluate the effectiveness of 16 materials in protecting cattle from horse flies. One material (ENT-25624) was almost completely effective for 8 hours and provided a high degree of protection for 32 hours but was ineffective after 48 hours. None of the other materials were highly effective after 6-8 hours.

8. Lice. In Mississippi, 9 promising insecticides were evaluated by the "spot-test" method against cattle lice. Five of the insecticides gave 100% control of existing infestations but none showed residual effectiveness beyond 7-9 days. In feeding tests, menazon at 10-15 mg/kg daily for 3 days gave over 95% control of motile lice. Continuous feeding at this rate might eliminate louse infestations.

9. Ticks. In field tests in Texas conventional sprays of 0.1% Shell Compound 4072, 0.75% Dowco 175 and 0.5% toxaphene (standard) were equally effective in controlling the lone star tick. Conventional spray applications of 1 gallon of 0.5% toxaphene were no more effective than 0.38 gallons applied by a spray-dip machine. None of the treatments prevented reinfestations longer than a week or 10 days.

In field tests against the winter tick, toxaphene at 0.5%, Dowco 175 at 0.75% and Ciodrin at 0.3% gave equally effective immediate control. In comparative tests, applications of 0.5% toxaphene as conventional sprays (1 gal/animal) and by spray-dip machine (0.38 gal/animal) were equally effective.

Field tests against Dermacentor andersoni on cattle were made in Wyoming with recommended sprays (1 gallon per head) of toxaphene (0.5%), coumaphos (0.5%), and dioxathion (0.125%). Toxaphene was also tested alone and in combination with lanolin, Aroclor 5460 (ENT-2589), and other potential extenders at 5%, a dosage which is ten times the recommended toxaphene concentration. However,

only 378 ml (instead of 1 gallon) were used and the material sprayed only on the lower neck and brisket area, where the ticks concentrated. With the standard treatments, tick reductions for toxaphene, dioxathion, and coumaphos were 55, 71, and 91% respectively, after 7 days, and 21, 23, and 57% after 14 days. Toxaphene alone at 5% (378 ml) showed 98, 86, and 20% reductions after 7, 14, and 21 days, respectively. Toxaphene plus four extenders gave reductions of 98-100, 86-97, and 4-50% after 7, 14, and 21 days, respectively. Results of these tests indicated that toxaphene is more effective than either dioxathion or coumaphos and also that concentrated sprays of toxaphene with additives applied to a limited area on cattle provide greater residual effects than conventional sprays.

In tests in Mexico, sprays of 0.1% and higher of Shell Compound 4072 gave 100% kill of all ticks (Boophilus microplus and Amblyomma cajennense). Sprays of 0.25% coumaphos gave 100% kill of males but were slightly less effective against female ticks. Some molting nymphs survived all treatments and became adults. A few eggs were laid by 1 of 3 females from cattle treated with 0.05% Shell Compound 4072 but none hatched. One of 19 females from cattle sprayed with 0.25% coumaphos laid a few eggs but larval ticks did not emerge.

In Texas, 19 formulations of 16 insecticides were evaluated for control of the spinose ear tick in cattle. All of the materials and formulations gave excellent immediate control of ear ticks. However, after one month the most effective treatments were coumaphos, 5% dust (93-96%) followed by 0.25% coumaphos spray (77-81%). The only other treatment showing over 50% control after one month was 0.1% Shell Compound 4072 in a spray.

C. Insecticide Residue Determinations

1. Residue studies: In Texas, gas chromatographic methods were perfected for the determination of Shell Compound 4072 in animal tissues and milk. Analyses of tissues of animals 7 days after being sprayed with 0.25% of Shell Compound 4072 showed residues of 0.085 ppm only in the omental fat. None could be detected after 28 days. In a cooperative experiment with the Agricultural Engineering Research Division, two cows were sprayed with Shell Compound 4072. One was sprayed once in the conventional manner with 2 quarts of 0.15% Shell Compound 4072 and the other was sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.10% Shell Compound 4072. Milk samples were collected from both cows for 15 days. Milk from the cows sprayed by the conventional method contained 0.142 ppm of Shell Compound 4072 four hours after spraying, .0095 ppm at two days and less than .0003 ppm at eight days after spraying. The level of Shell Compound 4072 residue in the milk from the cows sprayed daily with the automatic mist sprayer ranged from 0.0010 to 0.0003 ppm throughout the observation period.

A method proposed by Langlois, B. E., et al. (1964. Clean up of dairy products for analysis of chlorinated insecticide residues by electroncapture chromatography. Ag. and Food Chem., Vol. 12, p. 243.) has been used with

minor modifications for the rapid extraction and clean-up of DDT from milk, followed by gas chromatographic determination. Milk samples were analyzed from two cows, one of which was sprayed once in the conventional manner with 2 quarts of 0.5% DDT and the other sprayed daily with an automatic mist sprayer that delivered 90 ml of 0.05% DDT spray. Milk samples were taken from the combined morning and evening production. The level of DDT in the milk from the cow sprayed daily with the mist sprayer during a 24-day period never exceeded 0.0045 ppm. The level of DDT in the milk from the cow sprayed once in the conventional manner reached a peak of 0.61 ppm 3 days after treatment and fell after 14 days to 0.17 ppm., where it continued through the 21st day.

Two cows were sprayed with ronnel, one in the conventional manner, once with 2 quarts of 0.5% ronnel and the other daily with 80-120 ml of an 0.2% spray. After 14 days both cows were washed and held for one week, when analysis of the milk showed that both were free of residues. The experiment was repeated on the same cows but the treatments were reversed. Milk samples were collected for 21 days and analyzed. Residues of ronnel were below the limit of sensitivity of the analytical method. The highest levels of residues found in the milk were less than 0.2 ppm 1 or 2 days after application of the 0.5% spray.

Milk samples also were analyzed from two cows using back rubbers treated with 0.5 gallon of a 2% oil solution of ronnel. The cows were forced to walk under the back rubber at least four times a day for 28 days. Milk samples were taken for analysis at intervals during 28 days after use of the rubbers began. There appeared to be no residues in the milk resulting from the use of the back rubbers.

A cooperative experiment with Agricultural Engineering Research Division was set up to study the efficiency of a new automatic spraying device developed for the application of insecticides to livestock. A calf was sprayed twice with 5% coumaphos by passing through the automatic mist spray device. Hair samples were taken from various parts of the animal's body, extracted with chloroform, and analyzed flurometrically for coumaphos. The amount of the insecticide deposited on different parts of the body varied from 2.8 to 176.7 micrograms per cm^2 and averaging 51.5 micrograms per cm^2.

2. Toxicity Studies. Research was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

A study to determine the interactions of Vitamin A and phenothiazine drenches with coumaphos was reported for FY 1963. During FY 1964 studies on blood from the animals used in that study included the effects on the Vitamin E of plasma and the Vitamin A and carotene of plasma. There are no significant differences between treatment groups for Vitamin E or for carotene. Vitamin A and carotene values decreased throughout the test in all groups. Plasma Vitamin A was affected by two interactions of treatments. With contaminated

coumaphos animals fed normal diets had lower mean values than those fed additional Vitamin A, whereas those animals treated with normal coumaphos showed no differences in plasma Vitamin A, whether supplemented with A or not.

In animals treated with normal coumaphos the plasma Vitamin A was increased by drenching with phenothiazine/lead arsenate. In those cattle treated with contaminated coumaphos the plasma Vitamin A was lowered by drenching with phenothiazine/lead arsenate.

Atropine, the standard antidote for poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion, the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

A new oxime, TMB-4, has been considerably more effective than previously studied oximes in preventing death and hastening recovery of coumaphos-poisoned cattle. Pralidoxime as 2-PAM chloride (Protopam chloride), in high dosages, this year gave encouraging control of coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Cattle were poisoned by carbaryl, then treated with 2-PAM chloride and Promazine. Clinically, the signs of intoxication were markedly increased after the administration of the two drugs, indicating a potentiating effect of one or both.

Performance standards have been established for emulsions, but not for suspensions. Analyses of dips made with coumaphos, ronnel (Korlan) and Ciodrin were performed. Ronnel performed extremely well, maintaining its concentration precisely during the dipping of 65 sheep in a 600-gallon vat. Ciodrin was a complete failure, the concentration being reduced by more than 60% by the passage of 52 sheep through a 700-gallon vat. Coumaphos showed an essentially uniform tendency to increase in concentration, indicating that sheeps' wool was selectively absorbing more water than toxicant.

The use of present insect chemosterilants for the control of insects must be restricted because of their potential hazards. Although none of these materials are approved for use, studies were continued to determine the

hazards to livestock. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa; particularly the deleterious effect upon the tissues that form white blood cells.

Further studies have shown a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals and birds. Incubating chicken eggs injected with the chemosterilants--apholate, tepa, or metepa at various times showed a disconcerting number of defective chicks. Defects included shortened upper or lower beaks, crossed-beaks, absence of legs, curled and fused toes, herniation of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

In further tests with chemosterilants Jersey heifers were selected and divided into groups for treatment and controls. All were observed for 3 months to establish estrous cycles, then the principals were fed apholate daily at a dosage of 1.0 mg/kg. No effect of apholate upon the estrous cycle of the heifers was apparent at the end of 7 months. The heifers were then placed with a Hereford bull for breeding, the apholate feeding continuing at the same dosage. Effects upon implantation of the embryo and upon gestation are currently being observed.

A test was completed with a single survivor of a group of four sheep given 1.0 mg/kg of apholate daily. The test feeding was terminated after the sheep survived 759 daily doses. Principal effect of apholate on this sheep was a reduction of white blood cells and blood platelets. Recovery from these deficiencies has been very slow and is still under study.

Ewes and rams fed a dosage of 0.5 mg/kg of apholate were bred during the feeding period. Ovarian and testicular biopsy tissues did not show evidence of damage by apholate. The ewes lambed normally. White blood cell numbers were slightly reduced. The test was terminated after 494 daily doses had been administered.

A second study was designed to show hematologic and teratogenic (deformity producing) effects that might occur with the feeding of apholate. Rams and ewes were selected, placed on diets containing a dosage of 1.0 mg/kg of apholate and allowed to breed. Three of four test ewes, and both control ewes, delivered normal lambs. One test ewe delivered a deformed lamb.

The deformed lamb showed a lack of eyes and eye nerves, nose, and shortened upper jaw. There was no spleen and the liver was rudimentary in size. A mass outside the body resembled liver. The dam of this lamb had received approximately 189 daily doses of apholate at the time of conception and the lamb was delivered after 345 daily doses had been given.

The response of Brahman cattle to Ciodrin, coumaphos, dioxathion and Shell Compound 4072 was compared to the responses of cattle of Hereford or other European breeding. Each of the four compounds produced a different result.

Coumaphos and Ciodrin produced greater blood cholinesterase depression in Brahman cattle than in cattle of other breeding; Shell Compound 4072 and dioxathion had just the opposite effect.

Research has continued on the treatment of animals poisoned by organic phosphorus compounds. Various oximes were studied for their effectiveness alone or in combination with atropine. Pralidoxime chloride (Protopam chloride) showed good effectiveness alone and in combination with atropine, particularly when the dosage of pralidoxime was kept high and repeated. TMB-4, a relatively new compound, gave good results in the treatment of coumaphos poisoning, the most difficult, usually, to control.

D. Biological Control

1. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microsporidia were collected in June, 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1%.

Three of 21 lots of Orthopodomyia californica larvae collected in November, 1963 possessed the flagellate, Crithidia fasciculata which represents the first parasite noted in this remarkably parasite-free mosquito.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge. Identification of the pathogen and its relationship with the host is being undertaken.

A bacterium (Bacillus sphaericus Neide) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory tests. Limited field trials against mosquito larvae breeding (Culiseta incidens, C. Peus and Aedes sierrensis) in rock and tree holes were conducted by treating these with the bacteria. Water temperature ranged from 38° - 48° F. No effect of the bacteria on the larval population was noted. It is possible that the cool temperature prevented build up of the bacteria.

2. Horse Flies and Deer Flies. In Mississippi collections of tabanid larvae in typical habitats showed about 19% of the larvae to be infected with a species of Microsporidia. Healthy larvae readily became infected after feeding on diseased larvae. The organism concentrated in the fatty tissues but also invaded the muscle and salivary glands. Muscles became nonfunctional when heavily infested.

E. Insect Sterility, Attractants and Other New Approaches to Control.

1. Mosquitoes. Studies on chemosterilization of mosquitoes were continued at Gainesville, Fla. Twenty-two compounds were tested as sterilants against larvae of Aedes aegypti. Of these compounds only two caused sterility. In other tests hempa at 50 to 100 ppm gave 99% sterility; ENT-50664 caused complete sterility in the few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants showed three of these to be highly effective in causing sterility in this species.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Tests with sterile males and normal females of Aedes aegypti indicated that multiple matings of females with successful sperm transfer do not generally occur. However, when the males were sterilized as larvae multiple mating of females did occur, indicating an inability of these sterile males to satisfy the sperm complement necessary in the female spermathecae to inhibit subsequent matings.

Studies with Aedes aegypti mosquitoes and the chemosterilant, apholate, were conducted to determine if resistance to the sterilizing action of this compound could be developed through selection with sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants for this and other species of insects.

In Oregon, ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p. quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Florida research was continued on factors affecting the attraction of mosquitoes and research initiated on finding specific attractants for mosquitoes. No evidence has been found to show the presence of a chemical sex attractant in Aedes aegypti mosquitoes. Chemotactometer cages were developed to evaluate mosquito response to specific chemicals.

In Oregon, extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

2. Stable Fly. Tests at Beltsville, Md., indicated that stable flies were less attracted to light than either house flies or face flies. Daylight, blacklight BLB, and blacklight BL lamps attracted small percentages of flies in outdoor cages (16% of a test population) whereas, attraction was 43% and 53%, respectively, with house fly and face fly populations.

In Texas topical applications of tretamine at 1.0 ug/fly reduced oviposition by stable flies and no eggs hatched. An application of 4 µg/fly prevented oviposition.

3. Face Fly. In Nebraska a field study was initiated in April and continued to September to determine the effect of releases of sterilized males on natural face fly populations. The test area involved about 1 square mile of pasture harboring 25 cattle. The weekly releases of sterile flies averaged 1500 in April, increasing to 20,000 in July, 25,000 in August, and 30,000 in September. The early releases apparently retained the build up of face fly populations but had no control effect during the summer and early fall.

Tests at Beltsville, Md., in outdoor cages indicated that face flies were more attracted by black light than house flies or stable flies. Response of face flies to light occurred principally during periods of evening twilight. In the laboratory red (660 mµ), green (550 mµ), and blacklight (360 mµ) radiation attracted some face flies. A comparison of blacklight and red showed 65-70% of the flies attracted to the blacklight, but only 4% to the red.

4. Horn Fly. In Texas, releases of tepa sterilized male horn flies at a ratio of 3 to 1 to normal males reduced breeding by 60%. The reduction was 16% less than expected, indicating that the sterilized males were not fully competitive. In laboratory feeding tests 5 and 10 ppm of tepa completely prevented oviposition. Feeding of 1.0 ppm reduced oviposition and only 5% of the eggs hatched.

5. Screw-worm. In Texas, 22 of 158 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worm when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration. About 18 additional compounds were sufficiently promising in screening to warrant further testing. The sexual vigor and longevity of males sterilized with ENT-50106 or ENT-50450 were reduced but those of males treated with ENT-50716 or ENT-50842 were not affected.

Higher dosages (whether topical or oral) of the chemosterilant, metepa, are required to sterilize screw-worm flies than stable flies. This verifies conclusions drawn from 1962 studies in which screw-worm flies metabolized metepa faster than stable flies and the sterilizing dose was therefore assumed in 1962 to be higher for screw-worms than for stable flies.

When screw-worm cases occur at places more than 100 miles from the known overwintering zone, the question arises about the possibility of sterilized flies recovering from radiation effects. Special tests were therefore made with flies irradiated as 5-, 5 1/2-, and 6 1/2-day-old pupae with 6200 r. Observations of flies maintained for 22 days until 95% had died of old age showed no recovery of fertility. Cytological studies of the testes and ovaries of flies treated in this manner up to 31 days old showed a continued degeneration of both testes and ovaries, with no regeneration of germinal tissue. It seems positive, therefore, that the present method of irradiation produces permanently sterilized flies.

Futher cytological studies showed the effects of a chemosterilant, tretamine, and gamma irradiation in the screw-worms to be similar, except at the first level of meiosis. Radiation of screw-worm oocytes resulted in many chromosomal aberrations during the 1st and 2nd meiotic divisions of the newly laid eggs; treatment with tretamine, however, resulted in normal-appearing meiosis, followed by visible chromosome damage during cleavage in the embryo larva.

In Texas, approximately 90 chemicals and other materials were screened as screw-worm attractants. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. One of the ten materials, ENT-26926X, was highly attractive in some tests, but failed in others. A slightly detectable flowery odor suggested the presence of an impurity, believed to be ethyl isovalerate. Ethyl isovalerate synthesized at Mission and believed to be about 66% pure, was very attractive in several laboratory and field tests. Methyl isovalerate was less attractive. Most of the other 9 promising materials were choline derivatives. Several of these were highly attractive in laboratory and preliminary field tests. An attempt was made to locate pheromones in screw-worms. There was no evidence of a pheromone that would attract males to virgin females, but there was considerable evidence that there may be a pheromone produced by males that is attractive to virgin females.

6. House Fly. Research on the development of sterilization for the control of house flies has been continued. In Florida, screening of new compounds for sterilizing activity, secondary evaluation tests in the laboratory, field experiments, and basic cytological and histological studies have been continued. Of 338 new compounds screened, 49 sterilized house flies completely at one or more concentrations when fed to adults. Two new compounds, hempa and hemel, which sterilized house flies, were of particular interest because they represent a type of chemical structure not formerly known or shown to cause sterility. In tests with hempa, males were sterilized by feeding for 3 to 5 days on 1% to 2.5% hempa, but not after feeding for only 1 to 2 days. Sterilized males contained motile sperm and transferred motile sperm to the females. Males sterilized with hempa were competitive with normal males in mating with normal females.

Tests were conducted at three poultry farms in Hernando County, Fla., to evaluate the effectiveness of two chemosterilants, metepa and apholate, and an insecticide standard, trichlorfon, for the control of house flies. The

breeding site of the house flies was in manure under the poultry cages. Semi-weekly bait treatments of 1% of metepa, apholate, or trichlorfon in a sugar-water solution were applied to these droppings. Male and female flies were collected in the poultry houses for sterility determinations. The poultry house treated with metepa bait showed little reduction in the population for the first 2 weeks of treatment. Fly populations progressively decreased for the next 3 weeks, followed by a slight rise the following 2 weeks. At this time, there was such a drastic drop in the population that within 1 week no flies or breeding could be observed. The treatments were discontinued at this time. During the next 2.5 months only three flies were observed and they may have flown in or been carried from some other area. The male sterility was usually above 90%, and female sterility was often 100%. Sterility tests were discontinued after the 7th week because of the complete lack of adults in the test area. The fly population at the poultry house treated with apholate remained at a fairly constant level for the first 6 weeks of testing, after which a decrease was observed for 2 weeks, but the population increased thereafter and remained at the early posttreatment levels until the conclusion of the test. Sterility induced in both sexes was rapid and nearly complete. The fly densities at the poultry house treated with trichlorfon decreased immediately after treatment and remained at about the same level throughout the test period.

Experiments have been continued to determine whether a dosage of a chemosterilant too low to prevent complete hatching or adult emergence in the flies to which it was administered might, by the accumulation of genetic injuries, eventually reduce or eliminate reproduction after successive generations of exposure. The experimental colony in which successive generations were fed 0.01% of apholate in the adult food, is now in the F_{40} generation. The number of progeny from treated flies pupating in the first five generations was not substantially different from the standard, but then began to decline. Only 20% to 40% of the population were reaching the pupal stage by the 34th to 39th generations. When this colony was in the F_{22} generation, two groups of 100 pupae each were taken from the colony and two new colonies started. These were reared on regular fly food for 6 generations. The rate of pupation still varied from about 7% to 45%.

Tests were conducted to determine the effects of metepa on the chromosomes of adult male house flies. Broken chromosomes in the metaphase stage were observed in males that had been allowed to feed for 4 days on food treated with 0.5% metepa. In some males that had fed for 4 days on treated food the chromosomes in the prophase stage appeared unbroken but they stained atypically. To determine whether gamma irradiation also produced this effect, house flies in the late pupal stage were exposed to a sterilizing dosage of 2850r from a cobalt-60 source. Twenty-four to twenty-five hours after irradiation, when the adult males were 15 to 16 hours old, the testes were removed and squash preparations were made. Chromosomal damage obtained by irradiation was strikingly similar to that produced by metepa. Fragmentation ranged from mild to severe, with atypical staining of the nuclear material.

In large outdoor cage tests at Beltsville, Md., usually 40-64%, but never over 75%, of the house flies present in the cage were attracted to the

radiant energy emitted by daylight, blacklight BLB, and blacklight BL fluorescent lamps. Most of the flies which responded were attracted during the first night of exposure.

F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Mississippi and Texas, in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State experimental stations, to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. In Mississippi, tests involved the exposure of three groups of cattle to insect attack--one group continuously, one group at night (1 hour after sunset to 1 hour before sunrise), and one group in daytime (1 hour after sunrise to 1 hour before sunset). Each group was exposed daily with animals infected with anaplasmosis from June 5 to July 10. All of the continuously exposed animals developed the disease within 4-5 weeks. At least one and possibly two of the other groups also contracted the disease. However, the tests were considered inconclusive and will be repeated in an effort to obtain more precise information on the relative importance of night and day feeding insects in anaplasmosis transmission. During the period in which transmission presumably occurred in these tests the principal daytime biting species were the horse flies, T. vittiger schwardti, T. lineola and T. fuscicostatus, and the mosquito, P. confinnis. No horse flies were active at night so biting was confined to mosquitoes, principally P. confinnis and A. quadrimaculatus.

During the summer animal-baited and light traps were operated at night in the Mississippi Delta in an effort to correlate bovine anaplasmosis transmission in an area as a whole with the relative abundance and feeding activity of mosquitoes and horse flies by species. The predominant species of mosquitoes based on animal traps early in the summer were Aedes vexans (about 50%), Aedes sticticus (about 20%), Anopheles quadrimaculatus (about 10%), Culex erraticus (about 6%) and Psorophora confinnis (about 4%). Later in the summer the predominant species were A. quadrimaculatus (36%), C. erraticus (31%), Psorophora confinnis (21%) and A. vexans (8%). Mosquitoes were greatest on a tethered steer, considerably less on a steer in a trap, and least in a light trap. Over 5000 mosquitoes were estimated to have fed on the tethered steer during one 12-hour test period. The feeding of this number undoubtedly adversely affected the health and comfort of the animal.

In Texas, monthly surveys were continued to determine the identity of external parasites on infected (anaplasmosis) and clean herds of cattle. Light infestations of the lone star tick were noted in January, heavy infestations from April through July, and very few after early September. The winter tick appeared in November and was abundant during December and January, and almost nonexistent by March. The spinose ear tick was moderately abundant to abundant throughout the year. Horn flies appeared in April and were present until November. Cattle were sprayed periodically to keep the adult population at low to moderate levels. Light cattle louse

infestations were noted from October to April. As a result of prompt segregation of reactor cattle, an anaplasmosis-free herd of 75 cattle has been developed during the past several years.

At Beltsville, Md., transmission experiments and cytological studies with experimental vectors were continued in cooperation with the Animal Disease and Parasite Research Division. Colonization of the Pacific coast tick (Dermacentor occidentalis) has been accomplished and one hereditary transmission experiment has been conducted. The adults were allowed to feed on a calf during the clinical stages of anaplasmosis. The progeny of these adults were then tested on a susceptible splenectomized calf. Anaplasmosis was not transmitted; however, proof of susceptibility by challenge has not yet been completed. A single engorged Dermacentor andersoni female was received from the Department of Veterinary Science, University of Nevada. This specimen was taken from a deer that, on subinoculation of blood with a splenectomized calf, was infected with A. marginale. The larval progeny of this female were placed on a splenectomized calf for testing, but the larvae did not attach and feed and all the ticks were lost.

Cytological studies on vector species have been continued at Beltsville. Infected and non-infected D. andersoni and D. occidentalis have been prepared for electron microscopy, but tissue examinations have not been completed.

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AREA NO. 15. SHEEP AND GOAT INSECTS

Problem. Sheep and goats are attacked by a variety of insects and ticks that are responsible for losses of many millions of dollars annually in reduced weight gains, decreased production and quality of wool, and in deaths of animals from gross attacks and insect-borne diseases. Sheep keds are a particularly serious pest in the northern States and screw-worms in the southwestern States. Fleeceworms, lice, and ticks are important pests wherever sheep and goats are raised. Safer, more effective, nonresidue-forming insecticides are needed to combat these pests. There is a special need to develop systemic insecticides that when given at low levels in feed, salt, or water would effectively control pests of sheep and goats and thereby save growers the expense of rounding up and treating flocks several times a year. New approaches to control, including attractants, chemosterilants, and radiation, should be explored and developed for controlling certain pests, as was done for the screw-worm in the Southeast. The possibilities of controlling insect pests of sheep and goats with insect pathogens, parasites, and predators also need to be investigated. Additional basic studies on the biology of the insects involved are essential for the development of biological and sanitation measures for their control. Research is urgently needed to determine which insects other than sand flies transmit bluetongue and the role of insects and ticks in the spread of other diseases of sheep and goats.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of sheep and goats. Studies are conducted on the biology, physiology and nutrition of pests of sheep and goats, particularly the screw-worm and Culicoides gnats, with some attention to sheep keds and lice; on the nature of resistance to insecticides and on the length of time insecticides remain on animal skin and hair; and on the absorption, metabolism, degradation, excretion, and mechanism of action of insecticides on the insects. A program is underway to find new ways to control pests of sheep and goats, with special emphasis on chemosterilants, antimetabolites, attractants, and non-insecticidal materials. Efforts are being made to develop adult screw-worm attractants for determining the abundance of natural populations and for use in baits for control. Research is concerned with the development of more effective contact and systemic insecticides and with studies to devise sanitation or management procedures to minimize or prevent insect reproduction. Primary emphasis is given to the evaluation of new materials that leave small amounts of or no residues and to testing of formulations that will prolong effectiveness against insects and minimize toxicity hazards. Studies are conducted in cooperation with the Animal Disease and Parasite Research Division to determine the occurrence of residues in tissues of animals treated with insecticides. A limited program is being conducted on the

relationship of insects to diseases of sheep and goats, involving experimental transmission from diseased to healthy animals with various species of insects, and insect surveys in epidemic areas. Current studies are centered on the insect vectors of bluetongue disease of sheep in cooperation with the Animal Disease and Parasite Research Division. The research is conducted in major laboratories in Kerrville, Tex., and Corvallis, Oreg., and in satellite laboratories in Mission, Tex., and Denver, Colo. Investigations on the screw-worm were discontinued at Kerrville in September 1962 and moved to Mission, Tex., headquarters of the Southwest screw-worm eradication campaign. At the beginning of FY 1963 the bluetongue transmission research was transferred from Kerrville, Tex., to Denver, Colo.

The Federal scientific effort devoted to research in this area totals 4.9 professional man-years. Of this number, 1.9 is devoted to basic biology, physiology, and nutrition; 1.4 to insecticidal and sanitation control; 0.4 to insecticide residue determinations; 0.4 to insect sterility, attractants, and other new approaches to control; 0.6 to insect vectors of diseases; and 0.2 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

There is a limited program in the States on insects affecting sheep and goats. Research in progress on the control of the sheep nose bot and the sheep ked is providing information useful to the livestock industry. The association of sheep nose bots with disease incidence is being examined. Various new insecticides are being administered to determine their effectiveness in control. Studies of application methods are being performed to obtain more satisfactory results with reduced labor cost and increased treatment safety. General insect pests affecting other livestock as well as sheep and goats are receiving careful attention. Results of studies of the biologies and control of lice and various fly pests are applicable in most cases to all hosts.

There are 0.9 man-years dedicated by the States to research exclusively on sheep and goat insects.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. Screw-worm. Research on the screw-worm continued at Mission, Texas, in support of the sterile male release program. A line of traps along the Colorado and Concho Rivers was used to determine the flight range of sterile, laboratory reared screw-worm adults marked with P-32 or dyes. In the initial experiment in May and June 1963, over 1 million flies were released. The most flies were recaptured within 50 miles of the release point, but 1 was trapped at 65 miles, 4 at 80 miles, 2 at 110 miles, 2 at 140 miles, 1 at 165 miles, 1 at 170 miles, and 1 at 180 miles from the release point. These studies indicated that a barrier sterile fly release zone to keep

fertile flies from reaching Texas would need to extend at least 200 miles into Mexico. Additional studies showed that flights in hot weather (June and July) were relatively short and that maximum dispersal occurred in spring and early summer, and again in September and October.

Research was continued to find and develop genetically distinct strains of screw-worm flies. In one study, about 229,000 adults of the Florida strain were examined for inheritable characters. Breeding and backcrossing studies with candidate mutants showed the presence of several strains. Tretamine (a known chemical mutagen) and irradiation were used to induce mutations. In these studies, 23 new strains were produced. Six strains showed unusual larval characters, involving the number of spiracles or the spines on the 11th abdominal segment. All strains were studied for hardiness and competitiveness and 7 strains discarded when they showed no promise of ever becoming suitable for field use.

Through the SAG test, a technique reported previously for determining mating aggressiveness of sterilized or mutant screw-worm flies, it was determined that one strain of Texas screw-worms contained highly competitive males. Male flies produced in the plant operated by the Animal Disease Eradication Division on beef lung were as aggressive as males from the Entomology Research Division's research colony reared on the standard horse meat diet. Male flies reared in an experimental hydroponic medium were also equivalent in mating ability to flies reared on horse meat.

Studies were continued on the effects of desiccation, starvation, and age of flies at time of release on survival. In studies with a substrain of the original Florida strain of flies selected for resistance to starvation (food and water), adults of the 16th to 19th generations survived as well for 96 hours as the original strain survived for 56 hours. At about 50 hours of starvation, 50% of the unselected strain flies were dead, but only 0.3 to 1.7% of the 19th generation of the new substrain. Sexual aggressiveness of the males in the new substrain appeared normal until the 20th generation when a drop of aggressiveness occurred.

A possible taxonomic difference between laboratory-reared screw-worms and wild screw-worms has been found. Further studies are needed to determine whether this larval characteristic is statistically valid.

In studies of effects of low temperatures, young larvae were less tolerant and pupae 5- to 7-days old were more tolerant than mature larvae. Adults exposed to low temperatures showed greater tolerance at 1 to 2 hours of age, as compared with those 1 to 8 days of age. Some abnormal adults were produced when pupae were held at low temperature.

2. Biting Flies and Gnats. It was previously reported that snipe flies annoying sheep and other animals in the coast range of Oregon were of the genus Symphoromyia, and that a few larvae collected were believed to be of that genus. In the past year, 78 additional larvae were collected and 22

emerging flies tentatively identified as Symphoromyia atripes or near atripes. All the larvae were collected within about a dozen feet of water in well-drained, non-compacted biome; some were in moss-covered soil, some in soil under thick clumps of blackberry canes, and some around the bases of alders and willows. The maximum length of larvae collected was around 15 mm. Most larvae were full-grown when collected, but half-grown larvae were in samples taken in the latter half of June. First pupation occurred in the laboratory on May 13; emergence at 76°F. has been from 7 to 13 days after pupation, with a median from 9 to 10 days.

Symphoromyia commonly collected in the Nashville, Oreg. area run to atripes in existing keys, but probably most are not S. atripes, and should be designated S. atripes complex. First adults were collected on June 16 near Corvallis and alongside Upper Drift Creek about 8 miles from Waldport. In July, it was found that the females were attracted not only to the collector, but also to other Symphoromyia females. Caged, living, newly-collected females and the collector were about equally attractive to free females. Seven hundred thirty-one females were collected on July 31 without finding a male, and males have not been collected in previous years. The males of this genus are reported to form mating swarms, but none was seen. The females were sometimes observed crawling over the surface of the ground in known larval habitats, and it is suspected they may have been preparing to oviposit.

Extensive searching near Evanston, Wyo., failed to discover snipe fly larvae, despite the presence of a few adults. However, larvae were found in moss and top soil in a Bear River meadow in Utah (20 to 25 miles from Evanston, Wyo.) at an elevation of 9100 feet. Five larvae were found near the base of new spring growth of false hellebore, in the shade of tall willow bushes, within 3 feet of a small creek that flows into Bear River. The larvae were in the upper quarter inch of soil, directly under several species of flat-growing mosses that were less than 2 inches in height; one larva was found in each 3 or 4 square feet of shredded soil. Indications that mice, shrews, chipmunks, and pocket gophers had dug the soil in this snipe-fly breeding area suggest that these rodents play a role in natural control of snipe flies.

B. Insecticidal and Sanitation Control

1. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Fifty-two new compounds were screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium. None of the compounds were effective at 0.1 ppm but ENT-25612, ENT-25780, and Monsanto CP-40294 killed all the larvae in 24 hours at 1.0 ppm. In field tests in Mexico, cattle infested with 1-, 2-, 3-, and 4-day old screw-worm larvae were sprayed with 0.05, 0.1, 0.15, 0.2, and 0.25% Shell Compound 4072, or with 0.25% coumaphos (Co-ral) at 2 1/2 gallons per animal. One day after treatment, no live larvae were found at 0.2 and 0.25% Shell Compound 4072, but some of the

cattle were poisoned. A few larvae survived in cattle sprayed with coumaphos and lower concentrations of Shell Compound 4072. Shell Compound 4072 at 0.1% and higher provided screw-worm control superior to 0.25% coumaphos. In a field test in New Mexico, dusts of 5% coumaphos and ronnel (Korlan) failed to afford 100% control of 1- and 2-day old screw-worm larvae in wounds on cattle, when applied by automatic duster.

2. Lice. In Texas, two herds of freshly-sheared Angora goats on two ranches were sprayed in March and April with 0.1% Shell Compound 4072. Biting lice were heavy on both herds and sucking lice light on one before treatment. One herd of 230 adult goats and 168 kids were treated with 100 gallons of spray and another herd, 82 goats and 30 kids, with 25 gallons of spray. No live lice were found on either kids or goats 1 day or 1 week after treatment. By September both herds of goats were heavily to moderately infested again with biting lice and one was also lightly infested with sucking lice. The large herd was sprayed with 100 gallons of 0.3% Ciodrin. At 1 day after treatment, no live biting lice were seen, but there was no apparent reduction in sucking lice. However, after 1 week, no live lice were found. By March 1964, the herd was lightly infested with biting lice, but no sucking lice were noted. One group of 150 nannies were sprayed and 90 kids dipped with 0.03% diazinon suspension. Another group of 74 adult goats and 37 kids was treated in the same manner, using 0.03% diazinon emulsion. At 24 hours and 1 week posttreatment, no live lice were seen on the animals examined in either group. The other herd of 102 newly-sheared nannies and 38 kids was heavily infested with biting lice. The nannies were sprayed and the kids dipped with 0.15% Ciodrin. At the 24-hour and 1-week examinations, no live lice were found.

On a ranch near Harper, Tex., a flock of mutton and ewe sheep were found moderately to lightly infested with sheep biting lice, Bovicola ovis. Fifty-four muttons and 51 ewes, all in full fleece, were sprayed with 100 gallons of 0.13% Ciodrin at 250 psi. The sheep were poorly wetted by the treatment, but at 24 hours posttreatment, an estimated 50 to 75% of the lice were dead. At 1 week and 1 month after treatment, no live lice were found.

3. Ticks and Keds. In Oregon, a small flock of lambs lightly infested with sheep keds was treated with a 5% solution of barthrin in corn oil. Each lamb was treated with 12 ml. of the barthrin solution, applied at random with a pump oil can. Control was over 80% in 3 weeks.

C. Insecticide Residue Determinations

1. Residue Studies. In Texas, gas chromatographic methods were perfected for the determination of Shell Compound 4072 in animal tissues. Analyses of tissues of animals 7 days after being sprayed with 0.25% of Shell Compound 4072 showed residues of 0.085 ppm only in the omental fat. None could be detected after 28 days.

2. Toxicity Studies. Research was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

A study to determine the interactions of Vitamin A and phenothiazine/lead arsenate drenches with coumaphos was reported for FY 1963. During FY 1964 studies on blood from those animals included the effects on the Vitamin E of plasma and the Vitamin A and carotene of plasma. There are no significant differences between treatment groups for Vitamin E or for carotene. Vitamin A and carotene values decreased throughout the test in all groups. Plasma Vitamin A was affected by two interactions of treatments. With contaminated coumaphos (Co-ral), animals fed normal diets had lower mean values than those fed additional Vitamin A, whereas those animals treated with normal coumaphos showed no differences in plasma Vitamin A, whether supplemented with A or not.

Atropine, the standard antidote for poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion, the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

A new oxime, TMB4, has been considerably more effective than previously studied oximes in preventing death and hastening recovery of coumaphos-poisoned cattle.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Performance standards have been established for emulsions, but not for suspensions. Analyses of dips made with coumaphos, ronnel and Ciodrin were performed. Ronnel performed extremely well, maintaining its concentration precisely during the dipping of 65 sheep in a 600-gallon vat. Ciodrin was a complete failure, the concentration being reduced by more than 60% by the passage of 52 sheep through a 700-gallon vat. Coumaphos showed an essentially uniform tendency to increase in concentration, indicating that sheeps' wool was selectively absorbing more water than toxicant.

The use of present insect chemosterilants for the control of insects must be restricted because of their potential hazards. Although none of these materials are yet approved for use, studies were continued in sheep to

determine the hazards to livestock. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa, particularly the deleterious effect upon the tissues that form white blood cells.

Further studies have shown a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals. A lamb born to a ewe fed apholate showed a total lack of eyes and eye nerves, a lack of upper jaw and nose, and numerous other anatomic defects, not the least of which was a total failure to develop a spleen.

A test was completed with a single survivor of a group of four sheep given 1.0 mg/kg of apholate daily. This sheep survived 759 daily doses and the principal effect of apholate was a reduction of white blood cells and blood platelets. Recovery from these deficiencies has been very slow and is still under study.

Ewes and rams fed a dosage of 0.5 mg/kg of apholate were bred during the feeding period. Ovarian and testicular biopsy tissues did not show evidence of damage by apholate and the ewes lambed normally. White blood cell numbers were slightly reduced. The test was terminated after 494 daily doses had been administered.

A second study was designed to show hematologic and teratogenic (deformity producing) effects that might occur with the feeding of apholate. Rams and ewes were selected, placed on diets containing a dosage of 1.0 mg/kg of apholate and allowed to breed. Three of four test ewes, and both control ewes, delivered normal lambs. One test ewe delivered a deformed lamb.

The deformed lamb showed a lack of eyes and eye nerves, nose, and shortened upper jaw. There was no spleen and the liver was rudimentary in size. A mass outside the body resembled liver. The dam of this lamb had received approximately 189 daily doses of apholate at the time of conception and the lamb was delivered after 345 daily doses had been given.

Research has continued on the treatment of animals poisoned by organic phosphorus compounds. Various oximes were studied for their effectiveness alone or in combination with atropine. Pralidoxime chloride (Protopam chloride) showed good effectiveness alone and in combination with atropine, particularly when the dosage of pralidoxime was kept high and repeated. TMB4, a relatively new compound, gave good results in the treatment of coumaphos poisoning, usually the most difficult to control.

D. Insect Sterility, Attractants and Other New Approaches to Control

1. Screw-worm. In Texas 22 of 158 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worm when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration. About 18 additional compounds were sufficiently promising in screening to warrant further testing. The

sexual vigor and longevity of males sterilized with ENT-50106 or ENT-50450 were reduced but that of males treated with ENT-50716 or ENT-50842 was not affected.

Higher dosages (whether topical or oral) of the chemosterilant, metepa, are required to sterilize screw-worm flies than stable flies. This verifies conclusions drawn from 1962 studies in which screw-worm flies metabolized metepa faster than stable flies and the sterilizing dose was therefore assumed in 1962 to be higher for screw-worms than for stable flies.

When screw-worm cases occur at places more than 100 miles from the known overwintering zone, the question arises about the possibility of sterilized flies recovering from radiation effects. Special tests were therefore made with flies irradiated as 5-, 5 1/2-, and 6 1/2-day-old pupae with 6200 r. Observations of flies were maintained for 22 days until 95% had died of old age--but none recovered their fertility. Cytological studies of the testes and ovaries of flies treated in this manner up to 31 days old showed a continued degeneration of both testes and ovaries, with no regeneration of germinal tissue. It seems positive, therefore, that the present method of irradiation produces permanently sterilized flies.

Further cytological studies showed the effects of a chemosterilant, tretamine, and gamma irradiation in the screw-worms to be similar, except at the first level of meiosis. Radiation of screw-worm oocytes resulted in many chromosomal aberrations during the 1st and 2nd meiotic divisions of the newly laid eggs; treatment with tretamine, however, resulted in normal-appearing meiosis, followed by visible chromosome damage during cleavage in the embryo larva.

In Texas, approximately 90 chemicals and other materials were screened as screw-worm attractants. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. One of the ten materials, ENT-26926X was highly attractive in some tests, but failed in others. A slightly detectable flowery odor suggested the presence of an impurity, believed to be ethyl isovalerate. Ethyl isovalerate synthesized at Mission and believed to be about 66% pure, was very attractive in several laboratory and field tests. Methyl isovalerate was less attractive. Most of the other 9 promising materials were choline derivatives. Several of these were highly attractive in laboratory and preliminary field tests. An attempt was made to locate pheromones in screw-worms. There was no evidence of a pheromone that would attract males to virgin females, but there was considerable evidence that there may be a pheromone produced by males that is attractive to virgin females.

E. Insect Vectors of Diseases

1. Biting Flies and Gnats. Studies were continued in cooperation with the Denver, Colo., laboratory of the Animal Disease and Parasite Research Division, on the transmission of bluetongue disease of sheep. Attempts to transmit bluetongue from sheep to sheep with Culex tarsalis mosquitoes were unsuccessful. Suspensions of Culicoides variipennis and Culex

tarsalis placed on lamb kidney cell tissue culture monolayers did not cause cytopathological effects in the tissue cultures. Culicoides and C. tarsalis adults were inoculated with tissue culture fluid containing bluetongue virus without undue mortality of the test insects. When C. variipennis that had been intrathoracically inoculated with tissue culture virus were ground and suspended and added to clean tissue culture monolayers, no virus was recovered if the suspensions were added to the tissue culture on the first day. However, after incubation for 10 and 13 days in the Culicoides before adding to the clean tissue culture, the virus was recovered from the tissue culture. This indicated that the virus inoculated into the Culicoides actually multiplied in the insect. Further studies verified the multiplication of bluetongue virus in injected Culicoides; the amount of virus tissue culture fluid injected in a Culicoides variipennis adult was less than 0.0002 grams. It was also demonstrated that bluetongue virus multiplied in Culicoides following blood feeding on an infected sheep. After incubation for 8 days (intrathoracically inoculated adults), the strain of bluetongue virus adapted to chick embryos was transmitted from egg to egg.

Sheep-baited animal trap studies in Colorado and South Dakota indicated that Culicoides variipennis, Aedes nigromaculis, and Culiseta inornata commonly attacked sheep. The following were taken while engorging on a staked sheep: 1 Leptoconops sp., 1 Chrysops sp., and 1 species of Simuliidae. Other field studies in South Dakota verified the frequency of attack of sheep by C. variipennis, A. nigromaculis, and C. inornata, and found two other species that also commonly attacked sheep: Culex tarsalis and Psorophora signipennis. Near Hudson, Colo., in a severe bluetongue outbreak, the virus was isolated from both sheep and cattle and massive C. variipennis breeding was located nearby.

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AREA NO. 16. POULTRY INSECTS

Problem. Numerous species of insects, mites, and ticks are common pests of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox disease which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. Safer, more effective non-residue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combatting poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

USDA AND COOPERATIVE PROGRAM

A continuing study is underway in the Department involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on use of chemosterilants, antimetabolites, attractants, and non-insecticidal materials and methods. Current studies in this field are largely limited to house flies. They include investigations of physical and mechanical methods for controlling house flies being conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective insecticides for the control of poultry pests. New chemicals are screened in the laboratory for contact and residual toxicity to lice and mites attacking poultry and to house flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and mites on the poultry, and render the droppings toxic

to fly larvae. Efforts are also being given to methods of sanitation and management to control breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with State Experiment Stations and poultry raisers at Gainesville, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex. Additional research is conducted at Lake Charles, La.

The Federal scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to basic biology, physiology, and nutrition; 0.9 to insecticidal and sanitation control; 0.2 to insecticide residue determination; 0.6 to insect sterility, attractants and other new approaches to control; and 0.1 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The States are performing both basic and applied research on poultry insects. Studies are in progress to determine the distribution and amount of damage caused by the more than 50 external parasites of poultry in the various States. As the extent of injury is determined, harmful species are studied to obtain information on their life histories and ecology. For example, in the northern fowl mite, the rate of population increase, the incubation periods of the eggs and duration of the immature stages are being studied. The effects of various population levels in production and fertility of eggs are also under investigation. The development of laboratory rearing methods is a pre-requisite for much of this research.

Control studies include comparisons of insecticides. New materials are constantly being evaluated as replacements for chemicals to which some poultry pests have been resistant. Treatment methods range from feed additives, which prevent fly development in manure or provide systemic control of parasites on the birds to direct or area applications. Insecticide residue analyses are performed to determine concentrations of parent compounds and metabolic products at specific intervals following applications. Rates of degradation are determined by bioassay and chemical analyses in eggs and tissues of the birds.

Studies are in progress on the effects of insecticides and management practices on the natural enemies of pest flies. Cultural practices which favor the biological control agents are integrated with selective applications of insecticides to minimize or prevent the destruction of natural enemies, but still obtain maximum kills of pest flies.

Research on external parasites of poultry also includes detailed studies to identify vectors of diseases. Suspect vectors are reared and fed on infected birds and transferred to healthy ones. Life history studies are performed on many of these insects.

There are 3.5 man-years committed by the States to research on poultry insects.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. House Flies. At Gainesville, Fla., various methods of tagging house flies have been evaluated and developed to study biology, dispersion, and behavior. Studies have shown that an individual male may attempt to copulate with a female at least 6 times in a day. Releases of tagged house flies on Grand Turk Island revealed the following house fly biology, behavior and dispersal: Flies dispersed in all directions from where they were released. In some cases flies remained around the release point for 1 or 2 days; in others they dispersed within a day. Using tagged flies it was shown that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the population. In another study the ratio of untagged males to untagged females was 1.5:1. The total number of flies on the island was determined to be between 200,000 and 300,000. These represent present survivors of the chemosterilant bait applications which are being conducted on the island.

Studies have shown that emergence of house flies from the pupal stage exhibited circadian rhythm. A postulate has been made that emergence begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon, studies on the physiology and biology of house flies were continued. A physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Strains such as this should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

In Oregon extensive studies were continued on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). Vermiculite was preferable to wood shavings in the larval medium, increasing ease in handling, facilitating separation of pupae, and reducing mite infestations. A ball of 2-day-old alfalfa meal and water enclosed in a white muslin cloth is the basis for a new method of obtaining eggs. The moist ball is placed on filter paper in a petri dish; about one-fourth of the filter paper surface is available for oviposition to the flies which prefer it to the muslin. Eggs are easily removed and an average of 300,000 eggs are collected within a 4-hour period, twice weekly, by this method. In further studies, little house fly pupae survived refrigeration at 41°F for 20 days; emergence of adults from pupae held at this temperature for 32 days was only 70% as great and most of the flies were smaller in size. Adults emerged in good numbers from pupae held 24 hours at 23°F, but none emerged from pupae held at 23° for 4 days.

2. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F_3 generation. Field studies on the wild strain of quadrimaculatus in Central Florida showed this species to consistently and predominatly consist of young females (females in the process of laying their first batch of eggs). The only factor influencing the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58°F and 100% RH for seven days without decreasing their viability.

At Corvallis, Oreg., studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Willamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41° to 46°F. When the temperature approached 46°F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days (75°F), larval and pupal stages required 2-3 days each with little mortality noted (77°F). The maximum life spans of a male and female

were 49 and 55 days respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70°F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences. O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs at 2nd - 4th instar larvae. O. californica was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phraetophytes. Aedes sierrensis require fluctuating water levels for egg hatch. In California, Orthopodomyia californica females fed on cotton pads saturated with blood of chicken, rabbit, or man, diluted with 5% glucose. The females laid eggs when blood fed, but not when deprived of blood, indicating that blood is necessary for oviposition. However, another mosquito, Uranotaenia anhydor could not be induced to feed on chicken.

Studies have confirmed that the major factor responsible for DDT-resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

B. Insecticidal and Sanitation Control

1. House Flies. At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon and ronnel were superior to the malathion standard against both colonies, but diazinon was the better of the two against the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard malathion against the regular colony flies.

At Corvallis, Oreg., research has been continued on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF have shown that propyl DEF is an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion-resistant house flies degraded C^{14} malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus resistant house flies and was also effective as a synergist for parathion against resistant flies.

In Florida, 32 insecticides were tested as house fly larvicides in manure under caged poultry. One to 2 gallons of water emulsion of the insecticide were used per 1,000 ft^2 of manure, to give insecticide dosages from 100-200 mg/ft^2. Larval density was estimated by a new and highly accurate technique 1 day before and at intervals after treatment. Dicapthon caused complete mortality of the larvae within 7 days in the only test run with this insecticide (200 mg/ft^2). At 200 mg/ft^2, Shell Compound 4072 killed all the larvae and at 100 mg/ft^2, gave 90% control after 7 days. In another test at 200 mg and in 2 other tests at 100 mg/ft^2, the insecticide reduced fly breeding by only 13-42%. Ethion at 100 mg/ft^2 gave 90% control after 7 days. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control of the fly larvae.

Of three colonies of the little house fly maintained in Oregon, the one most recently colonized showed indications of the greatest resistance to malathion and heptachlor which had been used from time to time at the poultry house that was the source of the three strains. Although ronnel had also been used at the poultry houses, no more than 2-fold resistance to this chemical could be found in the colonies.

2. Mosquitoes. Studies were continued at Gainesville, Fla., on the development of insecticides for the control of mosquitoes. These studies included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

In field tests of airplane spray applications of water emulsions or oil solutions for adult mosquito control, Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 0.1 lb/acre and were more effective than malathion at the same application rate.

Testing of compounds to determine the systemic toxicity against mosquitoes has been continued. Thirty-five compounds previously tested for systemic action in rabbits against body lice, were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

At Corvallis, Oreg., fenthion applied in granular formulations as a mosquito larvicide in log ponds was effective for 7 to 11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

At Fresno, Calif., in field tests against Culex p. quinquefasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

3. Lice and Mites. In Oregon three new colonies of poultry parasites were established. One was the chicken body louse; the other two were the chicken mite and Norco (California) strain of the northern fowl mite. (A previously colonized strain of the northern fowl mite is the Hansen strain).

In screening tests in Texas 7 commercial compounds were highly effective, giving 100% control of lice on poultry for 28 to 35 days. The most effective, Monsanto CP-40294, Shell SD-8436 and SD-8448, and Stauffer R-5725, were effective in sprays at a concentration of 0.05%, as was the malathion standard. In field tests, a flock of about 800 white leghorn laying hens was pen-sprayed. Before treatment, 35 or 40 birds examined had moderate to heavy infestations of the chicken body louse and some birds also had light infestations of the wing louse. The hens were sprayed twice, about a week apart, using 10 gallons of 0.25% Ciodrin for each treatment. At the time of the second spraying, only 3 of 40 birds examined had light infestations (less than 5/bird); 1 week after the second spraying, no lice could be found. On each of 4 subsequent weekly inspections, the birds were devoid of lice. In the treatments an attempt was made to spray under and upper sides of the birds, but it was obvious that not all the birds were thoroughly wetted by the spray.

In Oregon, the Hansen strain of the northern fowl mite showed an LD-50 of 0.0072% for malathion; with 3 candidate malathion synergists, the LD-50's were one-fourth to one-thirteenth as large, demonstrating high efficiency on the part of the synergists. The Norco strain of the northern fowl mite has a long history of exposure to malathion; after 4 years of usage malathion failed to provide control at levels of malathion above the recommended rates of application. Tests showed the Norco strain to be 3 times more resistant to malathion than the Hansen strain,with an LD-50 of 0.021% for the Norco strain. This is equivalent to 5.2 $\mu g/in^2$ for the Hansen strain and 15.2 $\mu g/in^2$ for the Norco strain. A malathion synergist, S,S,S-triethyl tritiophosphate restored the effectiveness of malathion, but boosted the efficiency even more for the Hansen strain. Northern fowl mites from the Oregon State University turkey farm also showed malathion resistance (LD-50 was 0.01%). The LD-50 for malathion synergized with ENT-25812 showed a 50-fold increase in toxicity over malathion alone. The newly colonized strain of chicken mites from the Oregon State University poultry farm was assayed for susceptibility to malathion and an LD-50 of 0.004% was indicated in this preliminary test (7.2 $\mu g/in^2$).

The Extension Service pointed out the need for control of chiggers on rangelands at the time turkeys are put on range. Currently recommended chigger control insecticides include chlordane, lindane, or toxaphene, but these cannot be used on range areas utilized by poultry or livestock, as they would cause residues in the meat. Malathion at 0.5 to 1 lb/acre gives

excellent initial control of chiggers, but effectiveness lasts only about 2 weeks. If turkeys can be protected for chiggers for the first 2 weeks, they will not suffer the downgrading of carcasses due to lesions from chigger attacks. Malathion is currently recommended for use in poultry houses and direct on birds for lice and other mites and it is now registered for application to turkey ranges just prior to release of the birds.

C. Insecticide Residue Determinations

1. Toxicity Studies. Research was conducted in Texas in cooperation with the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

Atropine, the standard therapeutic antidote against poisoning by organophosphorus compounds, acts by opposing the stimulation resulting from accumulation of acetylcholine but does nothing to treat the basic biochemical lesion or the inhibition of the essential enzyme, cholinesterase. A need for an antidote that would reactivate inhibited cholinesterase has been recognized for many years. Various oximes have been proposed and have shown beneficial action together with specificity toward both compounds and species of animal. In previous studies, the oxime dosages employed did not seem useful against coumaphos poisoning.

Although carbamate insecticides inhibit cholinesterase, as do organic phosphorus compounds, the process is by carbamylation instead of phosphorylation. Laboratory animal studies indicated that oximes such as 2-PAM intensified the action of carbaryl instead of reversing the enzyme inhibition. Phenothiazine derivatives have some potentiating effects in organic phosphorus insecticide poisoning.

Studies were continued to determine the hazards of chemosterilants to poultry. Previous reports have emphasized the radiomimetic effect produced by apholate, tepa, and metepa; particularly the deleterious effect upon the tissues that form white blood cells. Further studies have shown a second effect, teratogenesis - that is, the production of monstrosities and defects in the young of animals and birds. Incubating chicken eggs injected with the chemosterilants--apholate, tepa, or metepa at various times showed a disconcerting number of defective chicks. Defects included shortened upper or lower beaks, crossed-beaks, absence of legs, curled and fused toes, herination of the brain, lack of eyes, schistosomus, and growth retardation. At high dosages the embryos died or did not begin development.

D. Biological Control

1. House Flies. A pathogenic fungus of the order Entomophthorales was found in the little house fly colonies in Oregon. About 85% of the adult flies were killed before the infected colonies could be isolated. Development of the fungus apparently requires about a week in the fly. Of interest is the fact that the fungus tolerates a wide range of relative humidities, from

30% to 70%. Attempts to infest house flies with this species of fungus were unsuccessful. After about 3 months colonization the fungus strain was lost in spite of attempts to maintain it. Attempts to culture the fungus on an artificial medium (potato-agar) were unsuccessful. The method that was successful for 3 months relied upon transferring infected flies to cages of clean Fannia canicularis.

2. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microspordia were collected in June 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1%.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge. Identification of the pathogen and its relationship with the host is being undertaken.

A bacterium (Bacillus sphaericus) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory tests. Limited field trials against mosquito larvae breeding (Culiseta incidens, C. peus and Aedes sierrensis) in rock and tree holes were conducted by treating these with the bacteria. Water temperature ranged from 38° - 48°F. No effect of the bacteria on the larval population was noted. It is possible that the cool temperature prevented build-up of the bacteria.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. House Flies. Tests were conducted at 3 poultry farms in Hernando County, Fla. to evaluate the effectiveness of 2 chemosterilants, metepa and apholate, for control of house flies in comparison with an insecticide, trichlorfon. Semiweekly bait treatments with 1% of the chemicals in sugar-water solution were applied to manure beneath the cages. Male and female flies were collected in the poultry house for sterility determination. The poultry house treated with metepa bait showed little fly reduction for the first 2 weeks after treatment, but the flies progressively decreased for the next 3 weeks. In the following 2 weeks, there was a slight rise in fly counts, after which there was such a drastic drop in fly production that within 1 week no flies or breeding could be observed, and the treatments were discontinued. During the next 2 1/2 months, only 3 flies were seen and they may have flown into the area or have been carried there. Male sterility was usually above 90% and the female sterility was often 100% until the 7th week, after which the sterility tests had to be discontinued for lack of flies. The fly population at the poultry house treated with the other chemosterilant, apholate, remained fairly low and constant for the first

6 weeks, then dropped for 2 weeks. However, the population increased slightly and remained at the early posttreatment levels for the rest of the test. Sterility induced in both sexes was rapid and nearly complete, and the lack of control was believed due to infiltration. The flies were not eradicated as they had been in the poultry house treated with metepa. Fly densities at the poultry house treated with insecticidal bait (trichlorfon) decreased immediately after treatment and remained at about the same low level throughout the test period.

In Oregon studies were continued with the little house fly. With adult flies, residues of tepa were more effective than metepa, which was more effective than apholate. Tepa was also more effective when fed to the flies. The minimum amount required in the diet was about 0.01%. In a residual treatment, tepa was effective as low as 0.1 mg/ft^2. Pupae dipped for 1 minute in metepa produced flies with no detectable sterility, even at a concentration of 5% metepa. In cage tests, blue strings (wool yarn) impregnated with 1% solutions of metepa caused considerable sterility of this fly, but higher dosages would be needed for full sterility. Very few eggs were deposited when the strings were treated with 5% metepa and there was no hatch. No eggs were deposited in cages with strings treated with 10% metepa solutions. The metepa-treated strings were not repellent to the flies since as many landed on treated strings as on control strings.

In other tests male little house flies were more susceptible to sterilization by metepa than females. Preliminary mating competitiveness studies with the little house fly exposed to residual treatments of metepa at 10 mg/ft^2 look encouraging.

Tests to discover attractants for the little house fly continued in Oregon. None of 45 compounds screened as attractants in the field caught more than 5 flies. By contrast, fish fillet flour caught 840, defatted liver caught 484, and fish flour caught 464 flies. A large percentage of the flies captured were females. Fish flour was the most attractive of five materials tested in the laboratory, capturing 96% of the flies caught. In further tests, whole fish flour, defatted liver, and the flour made from fish fillets were oviposition stimulants. In other tests, little house fly adults alighted on blue and red strings in preference to green, yellow, and white strings.

2. Mosquitoes. At Gainesville, Fla., 22 compounds were tested as sterilants against larvae of Aedes aegypti. Only two of these compounds caused sterility. In other tests hempa at 50 to 100 ppm gave 99% sterility and ENT-50664 caused complete sterility in a few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants showed three of these to be highly effective.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males

treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Studies were conducted with Aedes aegypti mosquitoes to determine if resistance to the sterilizing action of apholate could be developed through selection of sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults of other species of insects cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants.

In Oregon ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p. quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Oregon extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

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AREA NO. 17. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world, where troops and civilian personnel of the United States are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently interfere with farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes,sand flies, gnats, the imported fire ant, and similar pests, should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which, when taken orally, would repel or prevent insects from biting. Sanitation, habitat management, and other noninsecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control, including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving applied and basic research on insects affecting man, households, and industrial establishments, including mosquitoes, house flies, human lice, bed bugs, gnats, fleas, ticks, mites, and other pests of man, and on cockroaches, ants, and several other pests of importance in households and industrial establishments. Research

is directed toward the development of more effective insecticides and repellents and involves primary screening of chemicals in the laboratory and field evaluation of promising materials. Investigations are conducted on the nature of insect resistance to insecticides, on the mode of action of insecticides, on the effects of radiation and chemosterilants, on attractants, on factors affecting attraction of biting insects to humans, and on the factors affecting the effectiveness of repellents. Attention is also given to the development of sanitation and management procedures and to biological control methods for mosquitoes, house flies, cockroaches, and several other pests. The major portion of the program is conducted at Gainesville, Fla. The remainder of the work is done at Corvallis, Oreg., Fresno, Calif., and Lake Charles, La. Close cooperation and evaluation of research data and needs are maintained with the Defense Department through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 2 1/2 professional man-years have been transferred from the Defense Department to support research on chemosterilization of house flies. Cooperation is maintained with the World Health Organization on studies for developing new insecticides and other methods of control of insects affecting man. The World Health Organization provides financial support (1/2 professional man-year) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control. Studies are also conducted in cooperation with the Soil and Water Conservation Research Division of ARS and the Bureau of Vector Control, California State Department of Health, on soil and water management procedures to prevent mosquito production in western irrigated areas and in log ponds.

The Federal scientific effort devoted to research in this area totals 20.8 professional man-years. Of this number 3.8 is devoted to basic biology, physiology, and nutrition; 9.1 to insecticidal and sanitation control; 0.7 to biological control; 5.7 to insect sterility, attractants and other new approaches to control; 0.5 to the evaluation of equipment for insect detection and control; and 1.0 to program leadership.

Additional research (2 professional man-years) has been initiated under a grant of P.L. 480 funds (S9-ENT-3) to the Facultad de Agronomia, Universidad de la Republica, Uruguay, on "Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay."

Research has been initiated in Salisbury, Southern Rhodesia, Africa,under a Participating Agency Service Agreement between the Agency for International Development and the Agricultural Research Service and a Cooperative Agreement between the Agricultural Research Service and the Agricultural Research Council of Central Africa on the feasibility of the sterile-male technique for tsetse fly control.

PROGRAM OF STATE EXPERIMENT STATIONS

There is a well-diversified research program in this area in the States. Studies of insects affecting man are concerned largely with mosquitoes, flies, midges, and gnats. In many instances, results of research on livestock

provide information which may be used to reduce the incidence of pests which annoy or transmit human diseases.

Biological studies include species distribution and dissemination, feeding habits, host preferences, diapause, nature of breeding sites, and seasonal population fluctuations. Chemical control studies involve screening more effective, safer insecticides to replace those to which pests have developed resistance. Studies on attractants, repellents, baits, chemosterilants, and water and soil breeding site management are being performed. Natural enemies are being sought out and evaluated for their effectiveness preparatory to eventual increase and release if this proves feasible.

Research on household insects ranges from basic studies on the biochemistry and genetics of resistant pests to tests of new methods of control involving antimetabolites, fabric impregnation and persistant insecticides which are nontoxic to warm-blooded animals. Studies on structural pests, such as termites and wood-boring beetles include evaluation of soil pretreatments with insecticides, effects of fumigation and basic investigations of habits and environmental factors influencing the establishment of infestations.

Research on insects in industrial establishments is concerned largely with the control of stored products pests. The species responsible for grain losses are being identified and the development of populations in relation to environmental factors is being investigated. The interrelationships of insects and mold development are under investigation. Control studies include evaluations of the relative resistance of different varieties of grain to insect attack, fumigants, grain protectants, and ionizing radiation. The effectiveness of various parasites and predators is also being determined.

A total of 21.7 man-years are devoted to research in this area in the States.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies on mosquitoes have been continued to elucidate basic biology fundamental to development of new and effective control techniques. Extensive laboratory and field research has shown that males of a laboratory colony of Anopheles quadrimaculatus do not disperse as readily as do males of the wild type. Laboratory crosses accomplished through forced copulation of the laboratory and wild strains have developed a hybrid strain which mated readily in the laboratory in the F_3 generation. The wild strain of quadrimaculatus in Central Florida is consistently and predominantly comprised of young females (females in the process of laying their first batch of eggs). The only factor that influenced the size of the population was the availability of larval breeding sites. Eggs of this species of mosquito could be stored at 58° F and 100% RH for seven days without decreasing their viability.

Preliminary attempts to establish laboratory colonies of Aedes infirmatus, Psorophora ferox and Culex nigripalpus were unsuccessful. In the laboratory,

male eggs of Aedes aegypti hatched sooner than female eggs. The formula, NT = No XC-rt fitted the hatching data.

At Corvallis, Oreg., studies were continued on the biology and ecology of mosquitoes. The occurrence and distribution of Aedes increpitus in the Willamette Valley was followed by sampling areas in Suver community considered representative of larval breeding sites in the Willamette River from Eugene to Portland. All larval instars were found at weekly intervals from January through March 1964.

Laboratory studies in Oregon showed survival of Aedes sierrensis larvae for at least one year. Thirty-one individuals survived when maintained at temperatures of 41° to 46° F. When the temperature approached 46° F a few of the larvae pupated and adults emerged. At the end of the year all individuals pupated or emerged from pupae when removed to room temperatures.

In houses in Corvallis, Oreg., the following species of mosquitoes were collected while feeding on humans: Culex pipiens, Culex peus, Aedes sierrensis, Aedes increpitus, Aedes vexans, and Culiseta incidens.

Studies on mosquito biology were continued in California. Continuous rearing of Uranotaenia anhydor in the laboratory was accomplished. Eggs hatched in 2.5 to 3.5 days at 75° F, larval and pupal stages required 2-3 days each with little mortality noted at (77° F. The maximum life spans of male and females were 49 and 55 days, respectively. Mating occurred readily and the preovipositional period was 4-5 days at 70° F.

Studies have led to a much better understanding of the ecology of Orthopodomyia californica. Contrary to suggestions in the literature, there appears to be little opportunity for competition between O. californica and Aedes sierrensis because of their different ecological preferences. O. californica is not a rare species. Larval stages were collected every month of the year. Overwintering occurs as 2nd - 4th instar larvae. O. californica was associated with constant or decreasing water levels in tree holes of cottonwood and willow which are phraetophytes. Aedes sierrensis requires fluctuating water levels for egg hatch.

Evaluations of adults from field collected larvae of 9 species of mosquitoes revealed autogenous egg deposition by only one species - Aedes pullatus.

Studies have confirmed that the major factor responsible for DDT resistance in the mosquito, Culex tarsalis, is the increased ability to detoxify DDT by oxidative metabolism.

A new station was established at Lake Charles, La., to study the biology and control of salt-marsh and rice-field mosquitoes. The station was not established in time to produce research results for this report.

2. House Fly. At Gainesville, Fla., various methods of tagging house flies have been evaluated, developed, and used to study their biology, dispersion, and behavior. An individual male may attempt to copulate with a female at least 6 times in a day. Releases of tagged house flies on Grand Turk Island showed that the flies dispersed in all directions from locations where they were released. In some cases flies remained around the location for 1 or 2 days, in others they dispersed within a day. It was determined that grid counts in buildings on Grand Turk represented approximately 1 to 10% of the fly population. In another study the ratio of untagged males to untagged females in the buildings was 1.5:1. The total number of flies on the island was somewhere between 200,000 and 300,000 which number represents survivors of the chemosterilant bait applications being conducted on the island.

Studies have shown that emergence of house flies from the pupal stage exhibited ciradian rhythm. A postulate has been made that emergence begins 12 hours after the onset of darkness. However, this response can be modified by the light regime of the parent generation, but is not obscured in toto. Preliminary data indicate that house fly susceptibility to DDT and diazinon also exhibited a circadian rhythm.

In Oregon a physiological mutant strain of the house fly was isolated in which the majority of females emerge before any males appear. Such strains should prove useful in biological and chemosterilization studies.

Further characterizations have been made of enzymes responsible for organophosphate resistance in house flies. The mutant aliesterase responsible for organophosphate resistance is a simple esterase. Activity of the enzyme was not affected by any of a number of metal ions or by chelating agents. Using centrifugation techniques, 50-fold purification has been achieved. Breakdown of organophosphorus insecticides by the partially-purified enzyme is inhibited by organophosphate synergists such as DEF. Studies utilizing disc electrophoresis have shown distinct differences in proteins and esterases between several susceptible and insecticide resistant strains. Evidence for allelism of the genes controlling resistance to two organophosphorus insecticides has been obtained. Also research indicated that the major genes responsible for malathion, parathion, DDT, and Isolan resistance in house flies are all carried on the same chromosome and that genes for resistance to parathion and malathion are alleles.

3. Stable Fly. Surveys were begun in the Panama City, Fla., area to determine the principal breeding sites of the stable fly. Adult flies were found as far as 50 miles inland; however, they tended to congregate along the Gulf Coast or other large bodies of water. Breeding or larvae was found in two types of habitat: grasses washed up on the shores of bays and lakes and manure in or near livestock shelters. The highest densities of larvae were found in manure under caged chickens; the manure at the periphery of chicken houses with open sides seemed to be the most favored place.

4. Ants. Information on the distribution, biology, and economic importance of the fire ant, Solenopsis saevissima, in Uruguay has been provided in

reports from investigators working in that country under P.L. 480 project S9-ENT-3. Geographical distribution of this species and two related species was more accurately determined. Heavy rainfall decreased the density of all three species of ants, particularly Solenopsis saevissima richteri. The nests of this species that survived had lower numbers of inhabitants and there was almost complete absence of other inhabitants usually associated with the nests.

Laboratory studies in Uruguay have been continued on the biology of the fire ant. For the first time other nest inhabitants such as Pselaphidae, Collembola, and Acarina have been maintained. Laboratory and field observations indicate that Labauchena daguerri is a social and obligate parasite of the fire ant and studies on its biology and association with the fire ant have been undertaken. Labauchena daguerri has been maintained with fire ant colonies in the laboratory.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies at Gainesville, Fla., included screening of candidate chemicals against mosquito larvae and adults and laboratory and field evaluation of promising materials. In screening tests with Anopheles quadrimaculatus larvae, 70 of 226 compounds were considered effective enough to warrant further evaluation. In screening tests with Aedes taeniorhynchus adults 28 of 174 compounds were equal to or more effective than the standard insecticide, malathion.

Exploratory tests with insecticides of relatively low mammalian toxicity were continued in cisterns on Key West to determine their residual effectiveness. Chlorthion gave 100% kills of Anopheles quadrimaculatus and Aedes aegypti larvae for 28 days; American Cyanamid E.I.-52160 gave 98% to 100% mortality for 21 days; and dicapthon 98% to 100% mortality for 7-14 days.

Field tests of airplane spray applications of Bayer 41831, Bayer 39007, and malathion as water emulsions or oil solutions for adult mosquito control were conducted. Bayer 41831 and Bayer 39007 reduced the adult population of Aedes taeniorhynchus and A. sollicitans by 99% at an application rate of 1 lb/acre and were more effective than malathion at the same application rate.

Thirty-five compounds previously tested for systemic action in rabbits against body lice were evaluated in rabbits against Aedes aegypti mosquitoes. Three of these materials (Bayer 30468, Hercules 7845-C and Rhodia R.P. 9895) caused complete mortality to at least one lot of mosquitoes fed within 5 hours after treatment (25 to 100 mg/kg) without noticeably affecting the rabbits.

Laboratory testing of compounds applied to plywood panels and other types of surfaces have been continued to evaluate materials that may be effective substitutes for DDT and dieldrin to which many species of malaria-carrying anopheline mosquitoes have become resistant. Field tests of the more promising materials have been conducted in Arkansas in buildings naturally infested

Anopheles quadrimcaulatus. Single applications of Bayer 39007 or Union Carbide UC-10854 produced 99% to 100% reduction throughout the first summer. The former compound caused at least 96% and often 99% to 100% reduction more than a year after application while the latter compound was less effective during the second year. When applied to infested buildings, Upjohn U-17004 and Bayer 37344 also were effective, but less durable than the other two compounds. In contrast to laboratory results, carbaryl rarely eliminated all Anopheles from treated buildings after the first week and control deteriorated rapidly.

At Corvallis, Oreg., fenthion applied in granular formulation as a mosquito larvicide in log ponds was effective for 7 to 11 days. In laboratory tests, a series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant mosquitoes.

In field tests at Fresno, Calif., against Culex p. quinquefasciatus, dichlorvos (30%) resin cylinders were lethal to larvae and adults at distances of 2 to 5 feet.

2. House Fly. At Gainesville, Fla., tests were conducted with emulsions of 17 compounds against natural infestations of house fly larvae in manure under caged poultry. Application rates were 100-200 mg/ft^2, of the active ingredient, using 1-2 gallons of liquid spray for each 1,000 square feet of breeding area. In the only test run with dicapthon, larvae were completely eliminated within 7 days. Stauffer N-2404, Bayer 25141, and Shell Compound 4072 also killed all larvae in one test, but in a second test Stauffer N-2404 and Bayer 25141 had little, if any effect. In one other test at 200 mg and two at 100 mg, Shell Compound 4072 eventually reduced the larval infestation to 13% and 42%, respectively. Dimethoate, Bayer 39007, and Hooker HRS-1422 also produced considerable control in most of the tests. The remaining compounds were relatively ineffective.

Residual tests were conducted with emulsions of various insecticides against house flies in Florida dairy barns. All of the materials were applied at 100 mg/ft^2. Diazinon was included as a standard. The diazinon treatment failed after 1 day in the first test. In the second test, the reduction of flies ranged from 78% to 90% for 5 days, after which the treatment failed. Dimethoate ranged from 83% to 94% for 8 days in one test, and from 91% to 96% for 3 days in the second test, after which the treatments became ineffective. Ciodrin gave reductions from 70% to 90% for 1 week. Fenthion ranged from 80% to 85% control for 5 days in one test, and failed after 1 day in the second. Bayer 39007 exhibited a reduction of 75% the first day, 68% the second, and was ineffective on the 3rd day after application. Endosulfan was ineffective in the first test and failed after 1 day in the second. Shell Compound 4072 was ineffective at 1 day.

At Gainesville, Fla., selected toxicants were tested as contact sprays against house flies of the regular (susceptible) and/or Cradson (multi-resistant) colonies. Dimethoate was the most effective against both colonies. Diazinon

and ronnel were superior to the malathion standard against both colonies. Diazinon was the better of the two against the regular colony and slightly less effective than ronnel against the Cradson colony. Hercules 9326 and ENT-27160 compared favorably with the standard,malathion,against the regular colony flies.

At Corvallis, Oreg., research has been conducted on the development of synergists for overcoming house fly resistance to organophosphorus insecticides. Tests with ethyl, propyl and butyl DEF have shown that propyl DEF is an effective synergist for parathion against parathion-resistant house flies. A series of unsymmetrical esters of phosphoric acid contained materials highly effective as synergists for malathion against resistant house flies. In addition, various dialkyl analogs of parathion and malathion were effective synergists for the parent compounds. House flies selected for resistance with synergized malathion became resistant more slowly than flies selected with malathion only. Malathion-resistant house flies degraded C^{14} malathion 2 to 14 times and excreted metabolites 2 to 4 times more rapidly than susceptible flies. Salithion was effective as an insecticide against susceptible and organophosphorus-resistant house flies and was also effective as a synergist for parathion against resistant flies.

3. Stable Fly. At Gainesville, Fla., 141 compounds were evaluated as stable fly larvicides. The most outstanding materials were ethyl DDVP, Shell Compound 4072, Bayer 25141, and dichlorvos with LC-50's ranging from 0.21 ppm to 0.64 ppm. Other highly effective materials were phorate, Bayer 24498, Bayer 30237, Bayer 29952, Monsanto CP-7394, Monsanto CP-10613, Bayer 30750, Bayer 39007, Shell SD-8447, Shell SD-8949, Shell SD-8972, dimethoate, Ciodrin, and Bayer 22492, and Shell SD-8988, all of which had LC-50's ranging from 0.81 to 1.65 ppm.

Six of the promising larvicides were tested against natural populations of stable fly larvae in chicken manure under caged laying hens at dosages of 450 and 45 mg/ft^2 of surface. At 45 mg/ft^2, Bayer 39007 produced 97% to 100% control for 21 days and 94% on the 35th day; Bayer 25141 gave 99% to 100% control for 14 days but only 80% by the 21st day; dimethoate gave poor initial control (71% and 62% after 1 and 7 days) but complete control on the 14th and 21st days; and Ciodrin, dichlorvos,and trichlorfon produced 89% to 99% control within 24 hours after application but were losing effectivenss rapidly by the 7th day. Control after 35 days at both dosage levels was generally low and erratic on all the plots.

Ninety chemicals were evaluated as stable fly adulticides. Forty-eight of the compounds gave at least 80% mortality after 24 hours at a concentration of 0.25%. The most outstanding materials were Ciodrin, Pyrolan, Bayer 39007, Bayer 22684, Shell SD-4092, and Shell SD-3423 with LC-50's ranging from 0.005% to 0.029%. Other outstanding adulticides were Telodrin, Shell SD-3959, Famophos, and Bayer 37341, all of which gave LC-50's ranging from 0.032% to 0.045%.

4. Cockroaches. At Gainesville, Fla., 103 compounds were screened as contact sprays against normal German cockroaches. In these tests 74 were rated Class III or IV (75 to 100% mortality at 2.0%). Tested at lower concentrations, several organophosphorus and carbamate insecticides were equal to or better than the chlordane standard in knockdown and mortality. As a residual treatment a carbamate insecticide, MC-A-600, was more effective than chlordane against susceptible German roaches. A lacquer formulation of diazinon did not persist as long as an emulsion formulation as a residual treatment.

Trials were run with an electric fogger to determine the effectiveness of several insecticides against natural populations of German cockroaches. Fogging with malathion, DDVP, or synergized pyrethrins was not a satisfactory method for controlling cockroach infestations; however, it could be a valuable addition to the use of an acceptable residual application since it would cause insects to vacate their harborages and contact the residual treatment.

Cooperative studies between personnel of the Corvallis, Oreg., laboratory and D. G. Cochran of the Virginia Agricultural Experiment Station, Blacksburg, Va., were conducted on synergism of malathion against malathion-resistant German cockroaches. Previous studies in Oregon have shown that certan synergists can overcome resistance to malathion in the house fly, the mosquito Culex tarsalis, and the northern fowl mite. In the studies with cockroaches, malathion resistance of greater than 20-fold was reduced to less than 3-fold through the use of 1:1 combinations of malathion and DEF. Tri-o-cresyl phosphate was not effective as a synergist. This material potentiates the toxicity of malathion against mammals and mosquitoes where resistance is due to a modified level of carboxyesterase activity. It is not effective with house flies where resistance is due to a mutant aliphatic esterase. The results suggest that resistance to malathion in the German cockroach is controlled by a factor similar to that responsible for resistance to malathion in the house fly.

5. Body Lice. Research was continued at Gainesville, Fla., to develop more effective insecticides for the control of body lice. Of 136 compounds screened, 39 were rated Class IV A in toxicity and 11 Class IV A in speed of knockdown. Of materials tested as synergists for malathion none were effective. In patch tests, one compound was more effective than the standard insecticides, DDT and malathion, while in sleeve tests Bayer 39007 was slightly less effective than malathion. In other sleeve tests two malathion powders formulated for slow release were less effective than the standard malathion powders.

Studies with two analogs of DDT and DDT-resistant body lice indicated that DDT-resistance is not caused by dehydrochlorination or an increased rate of oxidative metabolism of DDT, mechanisms found to be an important factor in DDT-resistance with other insects.

Approximately 10 strains of body lice resistant to one or more insecticides are constantly maintained and selected for resistance to these materials.

Periodically these strains are assayed for increased or decreased levels of resistance.

Fourteen compounds were evaluated as oral systemics in rabbits against body lice. Hercules 7845-C at 5 mg/kg, Rhodia R. P. 9895 at 400 mg/kg, Rhodia R. P. 13072 at 200 mg/kg, and Rhodia R. P. 13378 at 200 mg/kg caused complete mortality to at least one lot of lice fed within 5 hours after treatment without noticeably affecting the rabbits. Geigy G-24027 at 100 mg/kg caused 88% mortality of lice within 1 hour after treatment but killed a rabbit within 2 hours. This compound caused no mortality of lice up to 5 hours after treatment at 10 mg/kg but killed a rabbit between 5 and 24 hours after treatment. Nine others caused no appreciable mortality of lice at the highest dosage tested.

6. Mites, Ticks, and Fleas. Screening tests were continued in Florida to find new insecticides effective against fleas. Of 40 compounds tested against oriental rat fleas, Stauffer B-9323, Stauffer B-9627, Stauffer N-4446, and Bayer 37289 were the most effective compounds, producting 95% to 100% mortalities for at least 24 weeks. Twenty-eight compounds were evaluated for systemic action against oriental rat fleas. Five compounds caused complete mortality of some lots of fleas feeding on treated guinea pigs in 1 to 5 hours after treatment.

7. Bed Bugs. At Gainesville, Fla., 41 compounds were screened against bed bugs for insecticidal activity by means of a standardized technique of exposure on impregnated filter papers. Three compounds, Stauffer B-9323, Shell SD-8211, and Shell SD-8530 were highly effective materials, giving 95% kill for 24-week aging period.

C. Biological Control

1. Mosquitoes. Cooperative studies in California have been conducted on biological control agents for mosquito larvae. Many larvae of Aedes ventrovittis and A. hexodontus and a few larvae of Aedes cataphylla and Culex tarsalis infected with microsporidia were collected in June 1963 near Tenaya Lake in Yosemite National Park. Although infected larvae of some species were relatively abundant, the infected portion of the population was estimated at less than 1 percent. Three of 21 lots of Orthopodomyia californica larvae collected in November 1963 possessed the flagellate, Crithidia fasciculata the first parasite noted in this remarkably parasite-free mosquito.

An epizootic of possibly a microsporidian (not of the genus Thelohania) was observed in several large swales in which Aedes ventrovittis larvae were present in large numbers. Many dead larvae were present and most larvae were visibly affected. Some larvae were pupating but in the laboratory most pupae died or the adults failed to emerge.

A bacterium (Bacillus sphaericus) prepared by the Bioferm Corporation has been evaluated with success against many mosquito species in laboratory

tests. Limited field trials against mosquito larvae, Culiseta incidens, C. peus, and Aedes sierrensis, breeding in rock and tree holes were conducted by treating the breeding areas with the bacterium. Water temperature ranged from 38° - 48° F. No effect of the bacterium on the larval population was noted. It is possible that the cool temperature prevented build up of the bacterium.

D. Insect Sterility, Attractants and Other New Approaches to Control

1. Mosquitoes. Studies on chemosterilization of mosquitoes were continued at Gainesville, Fla. Twenty-two compounds were tested against larvae of Aedes aegypti. Of these compounds only two caused sterility. Hempa at 50 to 100 ppm gave 99% sterility and ENT-50664 caused complete sterility in the few adults that survived the 5 ppm treatment. Feeding adult aegypti mosquitoes with seven candidate chemosterilants, showed three of these to be highly effectivein causing sterility in this species.

Aedes aegypti larvae and adults were treated with apholate and tepa to determine if the males would recover fertility after successive matings. Recovery of fertility was almost complete by the 4th mating with males treated as larvae with apholate. Males treated as larvae with tepa showed less recovery of fertility. There was no indication of recovery of fertility in males treated as adults with residues of tepa.

Studies with Aedes aegypti mosquitoes and apholate were conducted to determine if resistance to the sterilizing action of this compound could be developed through selection with sub-sterilizing dosages. Selections were made by exposing larvae in treated water. Two colonies of aegypti selected over 5 to 10 generations developed resistance to the sterilizing action of apholate. Whether resistance would develop by treating adults or other species of insects cannot be predicted, but results indicate the possibility of the development of resistance which must be considered in the development of chemosterilants.

In Oregon, ethylenimine, a breakdown product of tepa, at 10 ppm caused no mortality of larvae, but high mortality of emerging adults of Culex p. quinquefasciatus. A dosage of 14 ppm of tepa to a ground pool containing Culex peus prevented most adults from emerging.

In Florida research was continued on the development of personal-use and clothing repellents for mosquitoes. New compounds, formulations and storage-life were evaluated. Tests were also run to find materials that would be effective as spare repellents. Two compounds, senecioic acid and N, N-diethylseneciamide, alone and in mixtures were evaluated. The latter compound showed some space repellency; the former very little.

In Florida research was continued on factors affecting the attraction of mosquitoes to humans and research initiated on finding specific attractants for mosquitoes. No evidence has been found to show the presence of a chemical

sex attractant in Aedes aegypti. Chemotactometer cages were developed to evaluate mosquito response to specific chemicals and odors or emanations, particularly from human arms.

In Oregon extracts of both sexes of Culex tarsalis and C. quinquefasciatus showed little if any attraction to mosquitoes of the opposite sex, though in one test an ether extract of female C. quinquefasciatus provided sufficient attraction to males of that species to warrant further testing.

2. House Flies. In Florida research on the development of sterilization for the control of house flies has been continued. Of 338 new compounds screened, 49 sterilized house flies completely at one or more concentrations when fed to adults. Two new compounds, hempa and hemel, which sterilized house flies, were of particular interest because they represent a type of chemical structure not previously tested. In tests with hempa, males were sterilized by feeding for 3 to 5 days on 1% to 2.5% hempa, but not after feeding for only 1 to 2 days. Sterilized males contained motile sperm and transferred motile sperm to the females. Males sterilized with hempa were competitive with normal males in mating with normal females.

Studies on the application of chemosterilant baits to privies on islands in the Atlantic Missile Range for the eradication of house flies have been continued. A high level of sterility and a greater than 95% reduction of house flies was obtained on Grand Turk Island through the use of chemosterilant baits. However, complete eradication has not yet been achieved. The number of flies still present on the island, including a high percentage of those sterilized by the bait treatment, was determined to be between 200,000 to 300,000. Techniques are being developed to release sterile males on Grand Turk to effect complete eradication of house flies on the island.

Tests were conducted at three poultry farms in Hernando County, Fla., to evaluate the effectiveness of two chemosterilants, metepa and apholate, and an insecticide standard, trichlorfon, for the control of house flies breeding in manure under poultry cages. Treatments of 1% metepa, apholate, or trichlorfon in a sugar-water solution were applied semiweekly to the droppings. The poultry house treated with metepa bait showed little reduction in fly population for the first 2 weeks of treatment but then populations progressively decreased and after 8 weeks no flies or breeding could be observed; the treatments were discontinued. During the next 2.5 months only three flies were observed and they may have flown in or been carried in from some other area. The male sterility was usually above 90% and female sterility was often 100%. The fly population at the poultry house treated with apholate remained at a fairly constant and relatively low level for the first 6 weeks of testing, after which a decrease was observed for 2 weeks. Thereafter, the population increased and remained at the early posttreatment levels until the conclusion of the test. Sterility induced in both sexes was rapid and nearly complete and the lack of control was believed due to infiltration. The fly densities at the poultry house treated with trichlorfon decreased immediately after treatment and remained at about the same level throughout the test period.

Experiments have been continued to determine whether a dosage of a chemosterilant too low to effect complete sterility in the flies to which it was administered through several generations might, by the accumulation of genetic injuries, eventually reduce or eliminate reproduction. The experimental colony in which successive generations were fed 0.01% of apholate in the adult food, is now in the F_{40} generation. The number of progeny from treated flies pupating in the first five generations was not substantially different from the standard, but then began to decline. Only 20% to 40% of the population was reaching the pupal stage by the 34th to 39th generations. When this colony was in the F_{22} generation, two groups of 100 pupae each were taken from the colony to start new colonies. These were reared on regular fly food without the chemosterilant for 6 generations and the rate of pupation continued to vary from about 7% to 45%.

Tests were conducted to determine the effects of metepa on the chromosomes of adult male house flies. Broken chromosomes in the metephase stage were observed in some of the males that had been allowed to feed for 4 days on food treated with 0.5% metepa and in some males the chromosomes in the prophase stage appeared unbroken but they stained atypically. To determine whether gamma irradiation also produced this effect, house flies in the late pupal stage were exposed to a sterilizing dosage of 2850 r from a cobalt-60 source. Twenty-four to twenty-five hours after irradiation, when the adult males were 15 to 16 hours old, the testes were removed and squash preparations were made. Chromosomal damage obtained by irradiation was strikingly similar to that produced by metepa. Fragmentation ranged from mild to severe, with atypical staining of the nuclear material.

3. Eye Gnats. Tests were continued at Gainesville, Fla., on the sterilization of eye gnats with chemicals and gamma radiation. Concentrations of tepa, metepa or apholate of 0.1% and lower in the food of adults caused little mortality but a high degree of sterility. With gamma radiation, dosages of 3500 r to 5000 r caused sterility in adults and the fertility decreased as the dosage was increased. Treated gnats appeared normal except for their decreased fertility and mortality of treated gnats was never greater than 5% by the termination of the test.

Examination of ovaries and testes from chemosterilant-treated eye gnats showed size reduction of testes and degenerate and misshapen cells in the ovaries. However, motile sperm were present in testes and transmitted by treated males to females.

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AREA NO. 18. BEES AND OTHER POLLINATING INSECTS

Problem. The pollination of some 50 seed and fruit crops depends on an abundance of honey bees and other pollinating insects. Although certain wild bees help to pollinate cultivated crops, honey bees are estimated to account for three-fourths of the pollination by insects. Most growers are not fully aware of the importance of honey bees and the wild bees in the production of insect-pollinated crops. Therefore, research that leads to more efficient and economical production of honey bees is imperative to insure effective pollination of many crops and the economical production of honey.

A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists, engaged in basic studies and in research concerned with the application of known principles to the solution of crop pollination problems for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, California, and Wisconsin, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are cooperative with the Wisconsin and Arizona Experiment Stations, the Department of Apiculture at Ontario Agricultural College, Canada, the Abbott and Pfizer Laboratories, the Eastern Utilization Research and Development Division, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and

Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz., are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah and Wisconsin, and Agricultural Engineering Research Division of ARS. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops Research and Agricultural Engineering Research Divisions, ARS, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 21.6 professional man-years. Of this number 6.7 is devoted to breeding and management to improve productivity in honey bees; 4.7 to biology and control of diseases and pests of honey bees; 5.5 to behavior and utilization of honey bees and other insects in crop pollination; 2.7 to effect of pesticides and other farm practices on honey bees and other pollinating insects; and 2.0 to program leadership.

P. L. 480 grants total 10.75 man-years. Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (2.5 man-years) and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Sao Paulo, Brazil (3 man-years). Bee disease research under P. L. 480 funds is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3 man-years) and with the Instituto Nazionale di Apicoltura, Bologna, Italy (3/4 man-year). Wild bee pollination research is being conducted with the Faculty of Agriculture, Department of Agricultural Zoology, University of Cairo, Egypt (1 1/2 man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations have a good research program on bees and other insect pollinators. The studies on pollination cover honey bees, alkali bees, leaf cutter bees, bumblebees and other insect pollinators. The behavior, life history, morphology, taxonomy and ecology of pollinating insects, pollination of alfalfa and other legumes and fruits and vegetables as well as other plants, the attractiveness of the blooms to the bees, the effectiveness of different pollinators in terms of seed or fruit yield, and the more important pollinating insects in different areas and factors responsible for their effectiveness are being studied. Methods of protecting and increasing species of pollinators are being developed. Studies are being made to determine the effect of pesticide chemicals on pollinating insects. The nest, characteristics of nest sites and nest parasites of pollinating insects are being described. The biotic and abiotic factors affecting the establishment, maintenance of wild bee population and the climatic and other factors affecting bees visiting crops to pollinate them are being studied. The most economical numbers of colonies of honey bees per acre for honey production and the saturation point with reference to pollination for seed production are being determined.

Management procedures for encouraging wild bees as pollinators are being developed. Wild and cultivated curcubits are surveyed to determine the pollinating insects which visit them and the relative effectiveness of these insects as pollinators of these plants. The role of chemicals found in bee secretions as well as sounds which are used in normal communicative media in honey bee colonies and the use of colonies of honey bees for pollination including the effect of location, colony strength and training of bees on pollination are under study.

Bee genetics and resistance to disease are elucidated through physiological, ecological, behavioristic and genetic studies of the general characteristics in the honey bee including resistance and susceptibility to American foulbrood. Control of American and European foulbrood and Nosema by drugs and antibiotics and methods of feeding colonies are being studied, as, is favorable apiary management to control or to retard the development of disease.

The amino acid requirements of the honey bee are being studied in an effort to develop a pollen supplement or substitute. Nutritional factors responsible for the differentiation and maintenance of castes of honey bees are being investigated.

Methods of queen rearing of honey bees, and the study of natural and artificial mating with the purpose of improving artificial insemination are being studied.

There are 18.7 man-years devoted to research on bees and pollination at the State stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Breeding and Management of Honey Bees

1. New Stock Lines. Using methods developed by the Baton Rouge, La., laboratory to transport eggs, semen, and young larvae, 4 new lines of stock were obtained from Europe. The new stock originated in Greece, Anatolia, Yugoslavia, and Russia. It will be evaluated for future breeding usage. An embargo on the introduction of adult honey bees to prevent the introduction of Acarine disease has been in force for many years during which no new stock has been introduced. Transporting the immature stages introduces new stock without endangering the bee industry with Acarine disease.

Honey bee colonies showing a high percentage of pollen collected from alfalfa were selected for breeding studies at Baton Rouge, La. Lines have been established by brother-sister artificial matings from 3 of the highest pollen-collecting colonies of the previous year's selections and from 3 of the lowest collectors. The performance of these lines as alfalfa pollinators will be tested in Utah in 1964.

2. Genetic Studies. The performance of F_1 and F_2 hybrid queen combinations were compared for queen survival and colony build-up at the Baton Rouge, La., laboratory. In none of the character studies was there significant differences between the F_1 and F_2 generations. Distributing F_2 generation queens for use of the commercial queen producer in a hybrid program would be more economical and advantageous because these breeder queens would provide more larvae for grafting purposes.

Mating flight of drone honey bees occurs normally only during a limited period of the day. At Baton Rouge, La., during early June this was between 2:00 and 3:30 p.m. Earlier in the spring the normal flights were about 2 hours earlier. In general, as the temperature and day length increased mating flights occurred later in the day and tended to be of shorter duration. Confining drones in cool and darkness during their normal flight time one day resulted in earlier than normal flights the following day. The second day after confinement flight activity returned to the normal time for that season of the year.

Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (E21-ENT-7). The viability of brood in worker combs of inbred honey bee stock may be reduced to 50%. It was thought that the other eggs do not hatch. However, it was shown in this work that these "lethal" eggs do, in fact, hatch and are eaten by the nurse bees soon after hatching. When eggs in worker cells were transferred to drone cells only half of them survived. Similar transfers to queen cells also resulted in 50% survival. By using stock having genetic markers to inseminate queens and rearing the resulting larvae in incubators to the prepupa stage genetic proof was obtained that the (lethal) drone larvae were diploids. The eggs which hatched and are eaten by the nurse bees are drones produced from fertilized eggs (diploids). Drones which normally survive are produced from unfertilized eggs (haploids). When techniques can be developed to bring these diploid drones to adulthood, some exciting prospects in breeding work may be expected.

The technique developed for rearing worker bees in the laboratory is not sufficient for drones. Drone larvae need access of air to spiracles of both sides of the body. The best results were obtained when the drone larvae were put into tubes. A higher percentage of the larvae developed to the pupa stage when they were placed on a gauze substratum in 80% relative humidity.

Investigations on artificial insemination of queens showed that with one insemination, each drone contributes roughly to an equal degree to the progeny of the queen. But with two inseminations the progeny of the drones represented in the first insemination were in most cases most numerous.

Research on bee breeding is also being conducted at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (S3-ENT-1). One of the significant findings was that queen substance

stimulates ovary development in the workers of Trigona postica while it acts as an inhibitor in Apis workers.

3. Queen Storage. At Madison, Wis., overwintering caged queens in multiple queen storage colonies is a practical method of holding reserve queens. Old queens or virgins do not survive as well as young mated queens. Sixteen to 20 queens can be held in each colony to best advantage. Once accepted by the colonies, the survival of the queens depends upon a large population of young bees reared late in the fall, protected from Nosema infection by fumagillin in the stores. The caged queens and food supply of the colony are so organized that the winter cluster cannot move away from the queens during severe winter weather. Each queen is provided queen cage candy containing 2% royal jelly.

4. Hybrid Bees. At Madison, the (6ZX)(M1) hybrid produced 48 pounds more honey than other hybrids tested against it. The respective average honey yields obtained from the seven stocks (6ZX)(M1), (Z6)(M1), (ZX)(M1), (R2)(MDy), Rossman commercial, (R2)(M1), and (Adams II)(M1) were 260,212, 197, 195, 181, 153, and 137 pounds. The (ZX)(M1) hybrid, superior to others in previous years' tests, was not fairly represented in this test because of poor queens resulting from late, unfavorable rearing and mating conditions on Pelee Island. Both (6ZX)(M1) and (ZX)(M1) rank superior to all others on the basis of temperament, behavior, swarming tendency, use of bur comb, and fall weight of brood nests. Inbred stock of the latter two should be considered for release to commercial organizations qualified to produce hybrid queens.

At Madison, Wis., nine inbred lines have been maintained, breeder queens produced, pedigrees brought up to date, and the percentage of inbreeding determined for each. Many hybrid combinations of these inbreds were made for prescreening tests to determine their combinability and colony characteristics. Outcrossed inbred queens are difficult for commercial queen breeders to use. Preliminary tests of 3-way breeder queens (6Z)(X) to establish greater vigor in the breeders (grafting mothers) indicate this to be a sound practice. Lines 6 to Z are of similar stock origin, and (6ZX)(M1) stock proved equal to previous tests of (ZX)(M1). Backcrossing (ZX)(ZX) to obtain greater vigor in breeding queens is apparently less desirable because of segregation of characters among the progeny.

5. Honey Removal. Further studies were conducted at Madison, Wis., to explore new repellents and methods for removing bees from supers. Benzaldehyde, described as a repellent by G. F. Townsend at the Guelph, Ontario Agricultural College, was tried and compared with propionic anhydride and dilute acetic acid and found equally effective at temperatures ranging in the mid 80's. Butyric anhydride, purchased as a commercial product was also equally as effective for removing bees as propionic anhydride under usual summer conditions. These materials are being studied further to determine their effects on bee longevity or honey flavor.

The use of high-volume, low-pressure air blast was highly effective for removing bees from honey supers at Madison, Wis. The entire honey crop of 35,000 pounds was removed by this method, using an industrial vacuum cleaner and a portable electric generator. Trials of other materials have not improved on this method.

6. Watering Bees. At Madison, Wis., the use of synthetic sheet sponge floated in a tank was adapted for out-yard use by providing two large oil drums as water reserve and maintaining the tank level automatically by the outlet position. No bees can gain access into the tank, and sanitation is maintained by periodically sterilizing the sponges.

7. Honey Quality and Refrigeration. At Madison, Wis., honey stored for 2 years in the deep freeze (0 to -5° F) showed no change in color, flavor, or granulation. Similar samples stored at room temperature (60° to 80° F) showed considerable darkening and flavor change. Other samples held in refrigeration (35° to 40° F) granulated solidly, but had no noticeable color or flavor changes. Deep-freeze storage of honey is of significant practical value and should be considered by the industry.

B. Diseases and Pests of Honey Bees

1. Diagnostic Service. At Beltsville, Md., the total number of examinations for bee diseases during the year was 1,165, of which 852 were for brood diseases and 313 for adult diseases.

2. Mites. At Beltsville, Md., additional samples of bees infested with mites were received from the Philippine Islands where such infestations continue to be a problem. Samples from the Malolos, Bulacan, area showed infestations of Tropilaelaps clareae and Varroa jacobsoni, both of which apparently attack the brood, resulting in dead pupae or defective adults. Samples received from Ormoc City, Leyte, were only infested with Tropilaelaps clareae; and samples from Sampalocan, Cumba, Lipa City, were only infested with Varroa jacobsoni.

An Acarine disease survey made under P. L. 480 project A7-ENT-10 in India showed that 11 provinces have been surveyed and Acarine disease has been found in the Indian honey bee (Apis indica) colonies in only 3 states. To date Acarapis woodi mites have not been found in the giant honey bee (A. dorsata) or the dwarf honey bee (A. florea) colonies. The symptoms of disease caused by mites in A. indica are similar to those found in the European honey bee (A. mellifera) in Europe.

Biological studies conducted under P. L. 480 project E15-ENT-1 in Italy showed that the parasitic mites were not only able to infest the minute branches of the prothoracic tracheae of the adult honey bee, but also occupied the air sacs of the thorax and frequently the air sacs of the head, by migration through the air trunks passing through the neck. This widespread distribution inside the bee contrasts with the usual belief that

they confine themselves to the prothoracic tracheae. Necrosis of the surrounding muscles was also shown. The demonstrations show that there are many migrating forms of Acarapis woodi on the thorax, on the second abdominal segment, and the base of the wings at certain periods of the year.

3. American Foulbrood. Thirty-nine-year-old American foulbrood scale (dried remains of diseased larvae) material obtained from Professor Haseman was examined at Beltsville, Md., and found to contain Bacillus larvae spores that were both viable and virulent upon test with honey bees.

At Laramie, Wyo., among larvae individually "inoculated" at the age of 6 to 30 hours with a uniform dosage of Bacillus larvae spores, less American foulbrood (17%) occurred in brood reared in a colony gorged with tylosin, than in a colony gorged with sulphathiazole (41%), or in an untreated colony (44%), all having queens of a disease-resistant strain.

Of various artificial culture media tested at Laramie, the one most favorable for sporulation of Bacillus larvae was the yeast extract and soluble starch medium of Foster (1950)1/. Addition of various levels of thiamin failed to increase growth of this species.

4. European Foulbrood. At Laramie, Wyo., brood combs from European foulbrood diseased colonies stored for 1/2 to 3 years remained infective, producing disease in all of 16 package colonies installed on the combs, the disease becoming severe in 5 of them. Gorging with gallimycin sirup and dusting with terramycin in powdered sugar, reduced, but did not eliminate, infections.

At Madison, Wis., a new formulation of gallimycin identified as E92-19 is effective against European foulbrood when fed at the rate of 3.3 g/gal. (198 mg activity) as a gorging treatment three times at 4-day intervals. One gram of E92-19 has 60 mg of erythromycin activity.

5. Tests for Antibiotics. The use of drugs and antibiotics for the treatment of bee diseases has stimulated a need for simple and rapid methods of detecting these materials in market honey. One such technique, which shows considerable promise, has been developed at Beltsville, Md., by modifying a presently available commercial microbiological assay kit. This microbiological kit is a colorimetric assay for determining desired concentrations of antimicrobials by observing the color change in a small filter paper disc impregnated with spores of Bacillus subtilis, a reducible blue dye and a buffered medium.

1/ Foster, J. W., Hardwick, W. A., and Guirard, B. (1950) Antisporulation factors in complex organic media. 1. Growth and sporulation studies on Bacillus larvae. Jour. Bact. 59:468-470.

6. Nosema Disease. At Madison, Wis., Nosema disease is of great economic importance in bee management, especially in the production, shipment, and use of package bees and queens. Late fall feeding of sugar sirup containing 100 mg fumagillin per gallon was of significant value in delaying the build-up of Nosema in late winter and early spring. The use of candy rather than bulk sirup as a carrier of fumagillin appears feasible as a midwinter Nosema treatment when sirup cannot be fed. Results of controlled Nosema inoculations of attendant bees with queens indicate that the disease is not transmitted from the attendants to the queen to the extent anticipated.

At Madison, Wis., late fall feeding of fumagillin sirup (0.9 gal.) to overwintering colonies is beneficial in suppressing and delaying winter build up of Nosema. Initial and subsequent levels of Nosema infection in treated colonies are considerably lighter than in untreated colonies, based upon 13 years of winter samplings.

At Madison, twenty-seven package colonies received from the Southern states were inoculated with Nosema when installed on April 30. Treatment with Fumidil B in sugar sirup, powdered sugar, and in queen candy indicates a measure of control with each method in proportion to the consumption of medication. Queen candy containing Fumidil B (200 mg fumagillin/10 lb.) was effective as a treatment and preventive for Nosema disease in nuclei and in package bees when these units were sufficiently strong to consume the candy steadily.

At Madison, preliminary studies show that Nosema apis can be transmitted to the bumblebee (Bombus fervidus). Reinfection of Apis mellifera with the spores grown in Bombus fervidus is not positively established but is strongly indicated.

Infrared irradiation of live honey bees to kill Nosema apis was attempted at Madison. Apis mellifera can tolerate internal temperatures of 132° F. at which temperature Nosema spores appear to be altered. Further evaluation will determine whether this method might be useful on shipments of live bees.

At Laramie, Wyo., both tylosin lactate and Fumidil-B, at a dosage of 1/2 gram per gallon of sugar sirup, failed to prevent Nosema infection in individually inoculated adult bees kept caged in an incubator, but Fumidil-B at a dosage of 4 grams per gallon completely prevented infection.

At Laramie, adult bees emerging from brood inoculated with low dosages of Nosema spores were apparently free of infection, but a small percentage of these adults (about 3%) were infected within 2 weeks, although sister control bees remained free of Nosema. Sister bees inoculated as adults became 100% infected with Nosema, while uninoculated adults remained free of infection.

At Laramie, dusting honey bees clustered on their combs in early spring 3 times at weekly intervals with 5 grams Fumidil-B in 45 grams powdered sugar per colony reduced the percentage of bees infected, and greatly reduced the infections in individual bees of the protozoan diseases, Nosema and amoeba.

7. Alkali Bee Brood Disease. At Laramie, Wyo., Aspergillus flavus was isolated from most samples of diseased alkali bee prepupae. Certain other fungi and bacteria also were isolated. The relationship of these microorganisms to the disease is being studied.

C. Bee Behavior

Honey bees at Madison, Wis., began flight as early as one half hour before sunrise and ceased flight one half hour after sunset. The kind of forage plant, as well as light and temperature, was a prime factor influencing how early or late bees flew. Strength of colony had no effect on the time of flight but only on the numbers flying at a given time. Bees worked black locust (Robinia pseudoacacia) at low temperatures (50° to 60° F) early in the day but were not active on the clovers or alfalfa until afternoon when temperatures ranged between 80° to 90° F.

In November, a standard two-story colony was brought into the bee room at Logan, Utah. Neither dried, ground bee-collected pollen nor the feeding of a pollen-sugar sirup mixture in the colony stimulated the bees to collect pollen but it did stimulate brood rearing. Two weeks passed before pollen collection started. The bees did better at high temperatures from 85° to 90° F and low relative humidity of 10 to 20%, than at high temperatures and high relative humidity of 35 to 75%. It was possible to inspect and handle the bees very readily in the morning when the nighttime temperatures were lowered to 50° F. Ver little or no smoke was needed and the bees did not attempt to fly to the lights.

D. Utilization of Honey Bees and Other Insects in Crop Pollination

1. Safflower. At Tucson, Ariz., gila safflower was grown in cages with honey bees in 1962 to determine the value of the bees as cross-pollinating agents. The oil in the seed was analyzed in 1963. The seed from the bee cages yielded 3.5% more oil than was obtained from seed grown in cages where bees were excluded, a difference that was significant at the 5% level.

The colonies in the cages at Tucson were divided into 4 groups and in addition to a permanent water supply and the flowers in the cage they were fed as follows: Nothing, sugar-sirup only, sirup plus pollen supplement, and sirup plus pure pollen cake. No treatment maintained broodrearing at the 500-inch level present when the colonies were caged.

Twenty-six species of bees were collected on safflower at Tucson during the early summer flowering period. There was a distinct variation in the seasonal abundance of the different bees. Two Lasioglossum species appeared first. They were followed by Agopostemon and then Melissodes, with Megachile appearing in the final weeks of flowering. There were roughly 10 honey bees for every wild bee seen in periodic counts. The pollen-collecting honey bees visited the plants in the forenoon. Nectar collectors were present most of the day.

2. Cantaloup. Data were collected by the Tucson laboratory on 618 cantaloup flowers under observation for pollinator visitation at Yuma on 15 days between April 30 and June 7. Of this number, 1 of 73 bagged to prevent bee visitation developed into a marketable melon and 24 of 107 left for open pollination developed into marketable melons. Numerous bee visits, up to about a dozen, increased the chance that the flower would set and develop into a marketable melon. During the peak of bee activity (midmorning) there should be about one bee per each 10 feet of row when the plants average one female flower each.

3. Lipids in the Bee Diet. At Tucson, Ariz., neutral, esterified and free sterol content of the non-saponified lipids of intact and eviscerated bees caged at emergence for 7 days on various foods were compared with the same materials from newly-emerged bees. Conclusions derived from the analyses were: The composition of the body fat of bees is affected both quantitatively and qualitatively by the dietary fat; there is a large lipid turnover in bees during the first 7 days of adult life; honey bees require fat (fatty acids) for growth and development; the adult worker is selective in its use of pollen lipids; the adult bee diet is probably the major factor responsible for the type of fatty acids incorporated in depot triglycerides; honey bees can synthesize lipids from non-fat diets; cottonwood pollen lipids are either poorly utilized or not used at all by honey bees; tissue of bees fed dandelion pollen had 3 times more saponifiable fat than newly-emerged bees and almost 4 times more than bees fed unextracted or fat-extracted cottonwood pollen, and the lipid component of both pollen and adult worker bees contains sterols that "look" and "behave" like cholesterol.

4. Wild Bee Pollinators. At Logan, Utah, a large scale experiment was conducted from June through September, 1963, to study the attractiveness of various nesting materials to Megachile rotundata and the survival of the larvae to the overwintering stage. One hundred holes of each 29 treatments were provided in a randomized pattern in 4 locations. Attractiveness was based on the number of nesting holes filled. Mortality was judged by inspecting 60 nests from each treatment. Results from counts on attractiveness to basic nesting materials can be listed in order of decreasing acceptance: Drilled 4" x 4" blocks, grooved boards, embedded straws, polyurethane blocks, corrugated cardboard, non-embedded straws.

At Logan, mortality was caused by biotic and abiotic factors. Biotic mortality included parasitism in the egg stage by the wasp Sapyga (Sapygidae)

and the beetle Nemognatha (Meloidae) and in the prepupal stage by the wasp Monodontomerus (Chacidoidea). There was also predation and competition for food by the beetles Tribolium madens (Tenebrionidae) and Trogoderma (Dermestidae). It was impossible, however, to assess the mortality caused by these two genera because they also acted as simple scavengers in nests where mortality had already taken place, and in nests from which adult bees had emerged. Of the parasites, Monodontomerus was most abundant, followed by Sapyga and Nemognatha. In the overall figures these species were responsible for the following mortalities (in order listed above): 0.68%, 0.12%, and 0.01%. Although the overall biotic mortality for all locations was 0.81% it was as high as 5% in one locality. Even though mortalities differed at each locality, the overall biotic mortality varied less than 2%.

At Logan, because only 2 of the 4 locations possessed sufficient nesting to inspect 60 nests in each of 29 treatments, and 1963 data offered somewhat contradictory results of previous observations, the experiment has been broadened and will be repeated in 1964.

Biological observations were initiated on Colletes ciliatoides Stephen in August, 1963, when a large nesting aggregation was discovered adjacent to an alfalfa field near Delta, Utah. The nesting site was located on the south exposure of a deep, drainage ditch bank.

A provisional description of the nest architecture was made from a consolidation of fragmentary evidence procured from nests examined. Burrows and branches were typical colletid type. Cell excavation, burrow and cell linings, and cell shape were also colletid type. The pasty, orange provisions filled most of the cells. Pollen analyses taken from cells and bee's scopae proved to be dodder (Cuscuta). Eggs were attached to cell caps immediately adjacent to their original attachment to the cell's "neck".

Fourth instar, predefecating larvae were found head up in cells. As defecation begins, each larva makes a 360° turn through the long axis of the cell smearing feces on its complete inner lining. Within a few hours after defecation is completed, the larva again makes a 360° turn through the long axis of the cell moving its anal segment back and forth in the same manner as during the defecation process. There is, however, one major difference. A clear, filamentous, gelatinous material is excreted through the anus. By the time the larva has completed this second 360° turn, the fecal layer is completely varnished with the clear layer. The anal segment is retracted and the larva assumes a condition termed prepupa. The outer surface of the gelatinous, clear material which is in direct contact with the feces becomes infused with it upon drying and the inner surface remains clear and smooth. Since this clear material becomes an unbroken capsule surrounding the larva which produced it, the name cocoon should be applied to it.

At Logan, dissections of predefecating ciliatoides larvae revealed a group of malpighian tubules attached to the proctodeum immediately posterior to its union with the ventriculus. In addition, a pair of tubules possibly malpighian tubules, were attached to the proctodeum nearer to the anal opening. These are not present in honey bee larvae. This pair of tubules becomes flaccid and shriveled after the transparent material is passed through the anus and, consequently, must produce at least part of the cocoon material. One parasitic bee species, Epeolus pusillus Cresson, was found in a number of ciliatoides cells.

At Logan, a character or group of characters separating larvae of the same species were sought and found. These groups were cold conditioned and subsequently placed in incubation for development. Positive sexing of developed pupae and adults were then associated with character groupings of larvae. As more species were examined, different characters were found on larvae of different bee species. Finally, a structural difference was found on the ninth sternite of alkali bees which separated 100% of females from males. When other bees, including the honey bee, were examined, it was found that this one character separated the sexes also. Thus far a total of 22 species have been successfully sexed in the larval stadiums based on structural differences on the ninth sternite.

E. Effect of Pesticides on Bees

At Logan, Utah, toxicity of 8 pesticides to honey bee workers and female Megachile rotundata was compared in the laboratory. Topical applications of each material were applied to groups of 20 bees at each dosage level. Materials tested were Meta-Systox, trichlorfon, toxaphene, Bidrin, mevinphos, phosphamidon, dimethoate, and diazinon. Trends of relative toxicities were obtained. The same experiment using more and different materials will be continued through 1964.

At Beltsville, Md., tests with an exotoxin of Bacillus thuringiensis var. thuringiensis demonstrated the toxicity of this material to honey bee larvae and adults. The lowest concentration of the exotoxin (3.12 mg/10 ml) killed only 5.4% of the larvae after 12 days with nearly 95% emerging as adults, whereas the highest concentration (200 mg/10 ml) killed all but 10.8% which emerged as normal adults after 12 days. This contrasts with the mortality of adults in tests where the same high dose killed 97.6% in four days. This, of course, is a very high dose of exotoxin, and it is quite improbable that this dosage level would be attained in field applications under normal methods of usage.

An experiment by the Tucson, Ariz., laboratory in cooperation with personnel of the University of California at Riverside was conducted at Brawley, Calif., to determine the value of covering colonies of honey bees with wet burlap for protection from carbaryl sprayed on cotton. The field was sprayed by airplane at night between 10 and 11 p.m. under ideal treatment

conditions. Burlap covers were placed over the colonies the night of application. Some remained on the colonies for two complete days. The burlap was sprinkled with about 1/2 gallon of water per colony about once each hour during the daytime. Some colonies were in the treated field, some were 50 feet from it, and others were in a check field 3/4 mile away.

Caged bees placed in the field at fly-over time showed 100% mortality compared to no mortality in the check field. Maximum ambient temperatures during the test ranged from 112° to 118° F.

Damage caused by drift of disorganized water-carrying bees after the colonies were placed by the treated field, before the material was applied, was greater than the pesticide damage. Dead bees in traps on the open treated-over colonies increased for 2 days, then returned to normal, which indicated some, but not severe, damage. There was a slight increase in the number of dead bees in traps on treated-over colonies covered one day, but colonies covered 2 days showed no abnormal number of dead bees in the traps. Bee activity in the treated field dropped sharply and did not recover during the following three days after treatment. Nectar concentration and volume in the cotton extrafloral nectaries increased after the treatment, which indicated that bees were not collecting the nectar. Open brood or frames of bees were not measurably affected by the treatment. Good bee visitation was observed in other cottonfields in the area during the same period of observation.

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AREA NO. 19. ANALYSIS, SYNTHESIS, FORMULATION, AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides are at present the most rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in products used for foods or feeds, or adversely affect wildlife, beneficial insects and other desirable organisms. More effective formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man. Insect chemosterilants appear promising for use in insect control and their potentialities should be thoroughly explored.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving chemists, entomologists, and scientists of other specialized disciplines to discover and develop new and improved insect control chemicals and methods of applying them. Chemical research to discover, isolate, and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md.; components of the cotton plant that serve as attractants, essential nutrients, or that otherwise affect the boll weevil, are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. Chemical research on synthetic organic materials and formulations for insect control is carried on at Beltsville, Md.; Gainesville, Fla., and State College, Miss. Development of analytical methods for insecticide residues is carried on at Beltsville, Md.; Tifton, Ga.; Kerrville, Tex., and Yakima, Wash. (The work of a laboratory located at Vincennes, Ind., was transferred to Beltsville near the end of fiscal year 1964.) There is cooperation with the State Experiment Stations in the respective regions of these laboratories. Cooperative work with the States on insecticide residues is conducted in connection with the following Regional Research Projects: NC-19, Fundamental Problems Associated with the Accumulation of Pesticidal Chemicals in Soils; NC-33, Pesticide Residues on or in Food, Feed, and Forage Crops; NE-36, Determination of Pesticide

Residues on Raw Agricultural Commodities; NE-53, Transformation of Insecticides by Plants; S-22, Pesticide Residues on Plant and Animal Products and Soils; W-45, Pesticide Residues, Their Nature, Distribution, and Persistence in Plants, Animals, and Soils; and Interregional Project IR-4, Evaluation of Current Data and Needed Research to Obtain Clearance for Safe, Effective Chemicals for Minor Uses on Agricultural Products. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to Research in this area totals 39.4 professional man-years. Of this number 5.5 are devoted to products of natural origin as sources of insect control materials; 17.8 to development of synthetic organic materials and formulations for insect control; 4.1 to methods of analysis for insecticide residues; 2.0 to aerosols for insect control; 7.0 to biological evaluation of insect control chemicals; 1.0 to methods for control of insects in aircraft; and 2.0 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations have an extensive program on the analysis and determination of insecticide residues which includes some research on acaricide residues. Instrumental, chemical and biological determinations of insecticide residues are made. Residues of insecticides are determined in/on crops and animals and their products and in soils and water. Residues are determined periodically from the day the insecticide is applied through harvest and in some cases during processing or marketing. Studies are made of changes that occur in pesticides after they are applied to plants. These changes include both those on and in the plants. One station is studying the effect of the plant cuticular waxes on insecticides. The effects of insecticides in soils upon growth, flavor and yield of various crops and upon the chemical, physical and biological properties of soils are determined. Pesticide soil colloid interactions are being studied. The occurrence of pesticide residues in tissues of living animals is also being studied as well as in animal products. Poultry and eggs are being analyzed to determine if any pesticide residues can be found in them.

Research is in progress to determine the total fate of insecticides applied to crops. This includes the amounts and nature of the products disappearing by volatility, metabolic changes, translocation and other ways.

Research is in progress on the drift of insecticides. This is a very serious problem especially in intensive agricultural areas where different crops are grown in adjoining fields. Pesticide residues in milk are being determined. On-the-farm practices which may contribute to the occurrence of insecticide residues in milk are being studied. Work is going forward on the evaluation

of current data and needed research to obtain clearance for safe effective pesticide chemicals for minor uses on crops.

Insecticide residue analytical methods are being improved and adapted to various crops, products, soils and water. Cleanup of samples and methods of sampling crops and animal products are under study to obtain more reliable results with the minimum amount of work. Cooperative research is in progress on the standardization of methods of insecticide residue analysis.

The research on insecticide residues is organized into 5 regional and one interregional project. The coordination of the work through these projects is effective.

Some stations make laboratory evaluations of the potential of chemicals as insecticides and acaricides. These are often tested against resistant strains of insects. One station tests about 800 to 1,000 new compounds each year. These are received from more than 20 firms for evaluation of their insecticidal or acaricidal properties. Fungus products are tested for toxicity to arthropods.

Research is under way on insecticide formulations through the study of the physical and chemical properties of carriers and solvents used in preparation of insecticide concentrates. The effects on the stability, dispersion, application and coverage by toxicants and the leaching of insecticides from granular formulations are determined.

Contact, ingestion and fumigation effects of insecticides are determined. Interactions and compatibility studies are made. Potential hazards from fire and explosion of fumigants are investigated.

Total of 33.7 man-years of research is devoted by the State stations to this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. Investigation of the natural sex attractants of a number of different insect species of economic importance is in progress at Beltsville, Md. A procedure has been developed for isolating in pure form the sex attractant secreted by the female pink bollworm moth. While only a very small amount of the pure attractant has been obtained as yet, the preparation of a sufficient quantity to permit investigation of the chemical structure is under way. Crude extracts of the attractant prepared from the whole bodies of the female moths are being supplied to the Plant Pest Control Division for pink bollworm surveys in Arizona and to Entomology Research

Division entomologists who are investigating the potential of the attractant for control of this insect through annihilation of the male moths by trapping.

Extracts have been made of the sex attractants of the tobacco hornworm, banded cucumber beetle, southern armyworm, lesser peach tree borer, and codling moth. These extracts have been fractionated and the individual fractions have been submitted for biological testing to determine which of them contains the active constituents. Investigation has progressed farthest with the tobacco hornworm and banded cucumber beetle attractants. In cooperation with other agencies, extracts were prepared from carpenterworm moths for the Forest Service and oriental fruit moths for the Canadian Department of Agriculture.

Procedures were developed for making gyplure, the synthetic gypsy moth sex attractant, of high purity, free of significant contaminants, on a semi-pilot plant scale, and about 16 kilograms of it was produced for the Plant Pest Control Division. The physical and chemical properties of gyplure were determined, including its stability in different solvents and under various other conditions.

In the investigation of the American cockroach sex attractant, conditions under which the females produce the attractant are being studied in order to develop a quantitative behavioral assay and to increase the efficiency of collection of the attractant.

Extracts and fractions have been prepared from imported fire ants for bioassay in a study of the trail-marking substance secreted by these insects.

2. Materials of Plant Origin for Insect Control. Extracts and fractions were prepared at Beltsville from plants of several species reported to have activity against insects and were submitted for testing for insecticidal or insect attractant or repellent properties. Samples of these extracts also were sent to the National Institutes of Health for antitumor tests. In cooperation with NIH a further fractionation was made of the neutral fraction of Anona glabra ether extract which had shown antitumor activity.

At State College, Miss., substances in the cotton plant that stimulate feeding by the boll weevil are being studied. Both water and chloroform extracts from cotton squares stimulate feeding. The water extract on standing in a frozen condition gradually is degraded and loses this activity in a few days. Blanching the dried squares with boiling water inhibits the degradation. Investigation of the effect of pH on the activity of the blanched water extract showed that the weevils fed much more vigorously when the extract was made alkaline than under acidic conditions. A mixture of diluted water and chloroform extracts caused more feeding than either extract alone.

B. Development of Synthetic Organic Materials and Formulations for Insect Control

1. Preparation of Synthetic Organic Compounds for Testing as Insect Chemosterilants. A new group of chemosterilants for male insects has been discovered at Beltsville that appears to offer a significant advance in the safety of chemosterilant use. Representatives of these are hempa (hexamethylphosphoramide) and hemel (hexamethylmelamine), alkylamides related in structure to some of the aziridinyl chemosterilants but much less toxic to mammals. These new chemosterilants have thus far been shown to sterilize male house flies, mosquitoes, boll weevils, codling moths, and screw-worm flies. This discovery opens a promising new direction in research on insect control by chemical sterilization. A number of analogs of hempa and hemel have been synthesized and found to have chemosterilant activity.

The effect of pH on the sterilizing activity of the aziridinyl-type chemosterilants tepa and metepa was investigated. Both of these compounds are very sensitive to acids and decompose rapidly at low pH, but within the pH range 6-8 they showed no significant variation in activity. The degradative pathway of tepa and metepa was studied and appeared to be similar for the two compounds. A quantitative comparison of the sterilizing effect of tepa, metepa, and apholate on male house flies by injection of graduated concentrations showed that tepa was 4 times as effective as apholate and 12.5 times as effective as metepa. Radioactively labeled (C^{14}) tepa was synthesized for use in a study that was begun on the metabolism of tepa in insects.

At State College, Miss., the antifertility effectiveness of apholate on male boll weevils was determined.

At Gainesville, Fla., a chemical investigation of sterility in insects was initiated. In the course of this study a gas chromatographic procedure was developed for determining the hydrocarbons present in cockroach hemolymph.

2. Preparation of Synthetic Organic Compounds for Testing as Insecticides, Insect Attractants or Repellents, or Synergists. In the investigation at Beltsville of Mediterranean fruit fly attractants a fourth isomer of trimedlure was discovered. The four isomers have been isolated in pure form and have been submitted for attractancy tests. The stereochemistry of these isomers is being studied. Two of the isomers are liquid and two are solid; one of the solid isomers (m.p. 72° C.) is unattractive. Because of crystallization problems encountered when trimedlure is used during cold weather, the use of a special winter grade with most of the 72° m.p. isomer removed has been proposed to Plant Pest Control Division. A number of analogs of trimedlure and other types of esters have been synthesized in a search for still better Mediterranean fruit fly attractants for survey and quarantine use.

At Kerrville, Tex., a number of compounds, as well as extracts of animal and plant tissues, were prepared for testing as attractants for screw-worm flies. Certain choline derivatives have shown some attractancy.

Diethyl seneciamide has been somewhat effective as a space repellent for mosquitoes (i.e., repelling the insects from some distance without actual contact). Compounds of some other types that were synthesized and tested for this purpose were found much more effective, showing promise for the eventual development of a good space repellent.

The "carbon-skeleton chromatography" method developed for the determination of molecular configuration was modified to use a thermal conductivity detector and otherwise improved so that it can be applied to a wider range of compounds.

3. Formulations. Research on improved formulations of insect control materials was carried on at Beltsville, Md. Means of evaluating the flowability of dusting powder were investigated. A method was developed that comprises tumbling the dust in a cylinder under standardized conditions during observation of the flow through a standard sieve. Establishment of reference powders for relative flowability evaluation also was investigated.

In cooperation with industry samples of water-dispersible powders of DDT and of carbaryl were tested to determine whether they met the requirements of Federal specifications.

At the request of the Armed Forces Pest Control Board supplies of technical DDT, emulsifiable DDT concentrate, DDT dusts and wettable powders, and pyrethrum-sesame oil aerosols that had been stored for a number of years at Department of Defense warehouses in Georgia and California were examined to determine their condition and recommendations were made regarding their retention or disposal.

Assistance was given to the Food and Drug Administration's Division of Antibiotics on particle size evaluation of antibiotics.

At the Gainesville, Fla., laboratory formulations of a large number of compounds were prepared for biological testing against insects affecting man.

4. Testing of Respiratory Protective Devices. Testing of commercially available respirator cartridges and gas mask canisters for protection against pesticides was continued. At the request of the Pesticides Regulation Division tests were made of the available devices with various new pesticides proposed for registration. Manufacturers of respiratory equipment were advised on improvement of their products. During the year they placed several new or modified models on the market and withdrew some of the older ones with a limited range of protection against pesticides. A completely revised, up-to-date listing of respiratory devices for protection against pesticides

was prepared and published in the ARS series. The previous edition of this list had met with wide demand; by the time the new list was issued about 14,000 copies had been distributed and many requests to reproduce the list had been granted. In cooperation with a committee of the American Standards Association a preliminary draft of minimum standards for respiratory equipment for protection against pesticides was prepared.

C. Methods of Analysis for Insecticide Residues

A gas chromatographic method was developed for the determination of ronnel residues in milk and animal tissues.

A method that had been developed previously for the analysis of General Chemical 4072 in fat and other body tissues of cattle was modified to adapt it to milk.

A gas chromatographic method of analysis was developed for residues of Shell SD-8477 and its chlorine-containing hydrolysis product in corn plants and ears.

Colorimetric and gas chromatographic methods were developed for the analysis of residues of 1-bromochlordene and some of its metabolites in crops.

An improved thin-layer chromatographic procedure was developed for the detection of chlorinated insecticides in milk and is being used by the Agricultural Marketing Service.

The fluorometric method of analysis for terephthalic acid residues in chicken tissues which was developed at the request of the Animal Husbandry Research Division was further refined and completed.

Methods of cleanup of samples of various crops and animal tissues containing residues were improved to increase the accuracy of analysis for a number of different insecticides.

Among special analytical problems undertaken during the year was an investigation of waste products and sludge from an endrin manufacturing plant in relation to fish kills that occurred in the lower Mississippi River. Certain compounds present in the sludge were shown to be identical with substances found in samples of the dead fish by U. S. Public Health Service analysts.

D. Aerosols for Insect Control

Two types of electric aerosol machines (a pulse jet machine and a small, portable air-blast machine) and a thermal aerosol generator were tested for the application of dichlorvos aerosols in greenhouses. Effective control of aphids, whiteflies, mealybugs, and spider mites was obtained with all three

machines. The small air-blast machine, which uses a vacuum cleaner-type blower, also gave excellent results in applying combinations of synergized pyrethrum, malathion, and dichlorvos for cockroach control in buildings, and in sterilizing buildings with β-propiolactone.

A study of the effect of particle size and length of exposure time on the kill of house flies by allethrin aerosols showed that aerosols of 10 microns mass median diameter (m.m.d.) were most efficient at exposure times up to ½ hour. A 2-micron m.m.d. aerosol was more effective with exposures of 2 hours or more.

The cause of deterioration and rupture of containers by deet insect repellent pressurized spray packed by a commercial company for procurement by General Services Administration was investigated. The trouble was found to be due mainly to the presence of excess moisture in the product. The company recalled this product and replaced it with satisfactory material.

A number of pressurized spray formulations of leech repellent were prepared to test various additives for preventing rapid washing of the repellent from skin or clothing by water.

The effect of perfumes and masking odors for pyrethrum aerosols on the insecticide and on container metals and linings has been investigated. The most stable masking odors tested thus far are esters such as methyl salicylate. Some ethereal oils such as oil of citron, orange, or lemon peel and oil of pennyroyal and certain terpenes present in oils of pine, juniper, and citrus have been found quite stable with the pyrethrins.

An all-plastic hermetically sealed aerosol valve has been developed and will be incorporated in a proposed federal purchase description for concentrated allethrin or pyrethrum aerosols. The use of this valve and the smaller containers permitted by the concentrated formulas will result in savings in cost of the units and shipping weight and space.

At the request of the Pesticides Regulation Division, several commercial aerosol formulations were tested for flammability. Particle size determinations were made on a number of water-based, emulsion-type insecticide pressurized sprays.

E. Biological Evaluation of Chemicals for Insect Control

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and

Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 23 other locations by other Branches of the Division, throughout the United States and in Mexico, on 63 insect species and 8 mite species. Some of the materials tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other Government or private research agencies and by industry. These materials are also submitted for evaluation to the Stored Product Insects Research Branch, AMS, laboratory at Savannah, Ga., and the Plant Pest Control Division, Methods Improvement Laboratory at Gulfport, Miss., in cooperative research with the Entomology Research Division.

A new quarterly reporting system on the results of these evaluations of candidate insecticides and acaricides was developed during the year in which results are received quarterly from each laboratory and compiled into a comprehensive report for internal use in the Department. Seven hundred and seventy-two compounds and extracts of fractions of natural products were evaluated during the year. Approximately 15% of these materials showed high toxicity to at least one species of insect or mite.

2. Materials That Control the Activities of Insects Through Effects Other Than Death. At Beltsville, in mating studies on the American cockroach in connection with sex attractant research, it has been found that a male may mate as many as 15 times. The average number of matings per male was 7.8. Twenty-six percent of the matings occurred within 48 hours of a previous copulation, with the shortest interval being less than 24 hours. Observations are being continued with male and female American cockroaches to determine the conditions under which the female sex attractant is produced, in order to develop a quantitative behavioral assay and to increase the efficiency of the process used to obtain the sex attractant. Collection of the sex attractant of the brown cockroach (of the same genus as the American cockroach) has been under way for some time.

Investigation of the sex attractant produced by the female southern armyworm moth has been continued. The insects are being reared and extracts prepared from the females at the Brownsville, Tex., laboratory for work on the isolation and identification of the attractant by the Beltsville chemists.

Large-scale rearing of Cecropia moths is in progress at Beltsville to provide a source of insect juvenile hormone. This hormone prevents the development of insects to the mature stage and might have possibilities for control of insect pests. The material obtained from the moths is for use in a chemical investigation of the nature and composition of the hormone.

In connection with research on insect chemosterilants to prevent insect reproduction, metabolism studies of C^{14}-tepa were continued and the amount of intact tepa recovered at successive time intervals after treatment was determined. This phase of the research was temporarily interrupted due to the discovery that the dimethylamido analogs of tepa (hexamethylphosphoramide)

and of tretamine (hexamethylmelamine), although less potent than tepa or tretamine, were effective house fly chemosterilants. Since these analogs are less toxic their discovery opens up new approaches to the use of chemosterilants.

3. Aerosols and Space Sprays. Laboratory tests were carried out with a number of the new insecticides and synergists in aerosols or space sprays to evaluate their effectiveness against resistant and nonresistant flies or mosquitoes. An aerosol containing 1.0% dichlorvos and 0.4% pyrethrins was highly effective against resistant house flies and Aedes aegypti mosquitoes.

A resistant house fly strain (F58W) established at Beltsville in 1958 by mixing seven different wild strains has retained its resistance well and was accepted in December 1963 by the Chemical Specialties Manufacturers Association for use as an alternate standard strain in the Peet-Grady, aerosol, and pressurized space spray test methods.

Several aerosol formulations applied by thermal or electric aerosol machines were tested for cockroach control under practical conditions. A pyrethrin-malathion combination was the most effective, giving control for about 2 weeks.

In laboratory jar tests, residues of Ciodrin® gave complete kills of chlordane-resistant German cockroaches, but lost their effectiveness after 8 to 12 weeks of aging. One confidential compound was still effective after 44 weeks.

In repellency tests against cockroaches, solutions of N,N-dimethyloctanamide and N,N-dimethyldodecanamide were repellent to resistant German, American, and oriental cockroaches.

Of a considerable number of compounds screened in the laboratory against face flies, CELA S-1942 supplied by a German company appeared the most promising.

At the request of the General Services Administration several samples of pyrethrum extract that had been in storage were tested against house flies and found slightly less effective than a laboratory sample of the same concentration.

F. Methods for Control of Insects in Aircraft

Experiments with carbaryl micronized dusts to kill Japanese beetles in aircraft showed that 100% kill of the beetles could be obtained with dosages as low as 0.25 g. of carbaryl per 1,000 cu. ft. Tests were carried out out to measure the rate of settling of the dusts and the data obtained were furnished to the U. S. Public Health Service for use in determining whether or not there would be any health hazard from their use in aircraft disinsection.

In studies on the effect of increased and decreased atmospheric pressure on insects, including Madeira cockroaches, house flies, Japanese beetles, Mexican bean beetles, and confused flour beetles, it was found that the beetles could exist at pressures as high as 135 p.s.i.a. for several days. In a high vacuum (0.05 to 0.03 mm. Hg) a high mortality of all the species occurred within about an hour. In the tests in vacuum the insects lost 14 to 47% in weight and in the Madeira cockroach and Japanese beetle a drop in internal temperature from 26° to 10° C. was observed during an hour's exposure.

The effect of gamma radiation on Madeira cockroaches was investigated. Death occurred in about 13 hours after the insects had been subjected to continuous radiation of approximately 235 r per minute. The electrophysiological activity of the roaches was recorded during the exposures. Tests with "jolts" of gamma radiation gave some indication that such treatment could change the circadian rhythm of activity of the roaches. This would be of interest because at present only light and temperature are recognized cues for setting the "biological clocks" of such organisms.

The effect of light and dark periods on the biological rhythm of Madeira cockroaches was studied in experiments where the insects were exposed to alternating periods of 12 hours light and 12 hours dark or 45 minutes light and 45 minutes dark. The electrophysiological activity of the insects was recorded continuously under both sets of conditions. With both the 45-minute and the 12-hour light-dark cycles the roaches showed two peaks of high level activity per day, the first at dusk and the second 8 to 10 hours later.

An experiment to measure changes that might occur in the circadian rhythm of activity of Madeira cockroaches in an earth satellite has been proposed to the National Aeronautics and Space Administration. This insect would be a hardy, light weight, convenient experimental animal for the study of effects of environmental stresses encountered in space flights on biological rhythms. In considering this proposal NASA required that the experiment would remain operational for a 21-day period of orbiting in the satellite. A mixture of sucrose, glycerol, peptone, and water was developed which would provide adequate nourishment for the roaches for the period and also would maintain a suitable relative humidity in the roach container. The proposed experiment is now under consideration by NASA.

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AREA NO. 20. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no known immediate concern to agriculture or mankind, but thousands of species are known to be or are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized, so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA AND COOPERATIVE PROGRAM

This program of the Department is a long-continuing one involving insect taxonomists, and includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks and mites, and the application of results of this research to the problem of insect identification. The work is carried on to a limited extent at Beltsville, Md., but mostly in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Somewhat less active cooperation is maintained with various centers of taxonomic research in the United States and foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 29.0 professional man-years. Of this number, 7.9 are devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 13.2 to identification of insects, mites, and ticks; 6.9 to preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.0 to program leadership.

Research in this area is also conducted under seven P. L. 480 projects. Two of these are in South America, one (S9-ENT-6) in Uruguay provides for 1.5 professional man-years devoted to the classification of grasshoppers and the other (S5-ENT-2) in Colombia, has 2 man-years devoted to a study of

Drosophila classification. Three projects are operating in India, as follows: A7-ENT-24 provides 3 professional man-years for a systematic study of thrips; A7-ENT-28 provides 2.25 man-years for taxonomic studies of Mallophaga (biting lice); and A7-ENT-29 provides 2 man-years for a taxonomic study of Bruchidae (seed beetles). A project in Egypt (F4-ENT-2) provides 4 professional man-years for study of the insect fauna of Egypt. A project in Pakistan (A17-ENT-10) provides 1.5 man-years on leafhopper taxonomy.

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations in general maintain a collection of preserved specimens of insect pests and beneficial insects that are of economic importance in the State. They also maintain a general collection of insects which may contribute to taxonomic research. Both of these collections are used in the identification of insects that are collected in the State. At a number of State stations the work in insect taxonomy is combined with ecological and biological studies. This combines the information on the physical characteristics of the insect by which it is identified with information on behavior including emergence, mating, flight, host selection, oviposition, feeding, activity patterns and other information and often includes data on various stages of the insect.

The number of insect species is so large that no one individual can be familiar with all of them. In most instances, an individual is familiar with only a limited group. At the State stations the work on taxonomy is usually limited to one or two groups which are of special interest to members of the staff. In one instance, taxonomic and biological investigations are conducted on small parasitic wasps--Chalcidoidea. Taxonomic analysis and revision of this group are being based on morphology, host relationships and behavior. Microlepidoptera are being studied in a similar manner. In Hawaii, taxonomic research emphasizes the fruit flies of the Pacific and Oriental regions. In other states, neotropical mosquitoes, aphids, scale insects, and leafhoppers are being studied. In addition to the taxonomy of insects, some State stations are doing taxonomic work on mites with the work mainly on mites of economic importance in the State. Studies are being made of the chromosomal evolution in insects. The identification of insect pests is an important aspect of the survey of economic insects which is carried on annually by a number of states, usually in cooperation with U.S.D.A.

A total of 25.8 man-years is devoted to this area by the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

1. Hemipterous Insects. Many species of leafhoppers are important agricultural pests, either as primary plant feeders or as vectors of plant diseases. The fauna of America north of Mexico is moderately well known, but that of the Neotropics has not been studied adequately. Planned revisionary treat-

ments of various groups of Neotropical leafhoppers have resulted in the publication of 5 comprehensive papers that advance our knowledge of these insects.

2. Beetles. Immature stages of insects are accurately identified only after they are positively associated with adults of previously described species. However, it is reasonable to assume identity of examples of certain large, immature specimens from a circumscribed geographical area when the large-sized components of the fauna of the area are well known. By such a process, satisfactory application of names to striking examples of the beetle fauna of Jamaica has been accomplished.

3. Moths. A new species of the genus Synanthedon, to which the lesser peach tree borer belongs, was described from specimens collected in Ohio. The small moths, of the family Walshiidae, that occur in North America were exhaustively reviewed and the data obtained from the study utilized to bring the classification of the family up to date.

4. Grasshoppers. In a cooperative study with a South American entomologist, a contribution was made to knowledge of the grasshopper tribe Ommexechini. A key to the genera of the tribe will permit their ready separation. Three studies of California grasshoppers added new species to our western fauna and demonstrated means for identification of these species and closely related ones.

5. Two-winged Flies. Through detailed study of the male postabdomens of a series of flies, reared from mines in leaves of a range of vegetables, it was possible to improve the nomenclature of a widespread pest of truck crops in the Southeastern States. Contributions were also made to our knowledge of the taxonomy of other groups of plant-feeding flies.

6. Thrips. A pine-inhabiting, predaceous thrips has previously been misidentified in publications, as Leptothrips mali (Fitch). This name applies actually to a species that is more or less peculiar to apple. The correct name for the predaceous thrips on pine is Leptothrips pini (Watson). When Watson described pini, he mistakenly placed it in a genus of fungus-feeding thrips. By transferring the species to Leptothrips, the true relationships of the species with other predaceous thrips are evident.

7. Bees and Wasps. Basic information has been gathered on the habits of various groups of solitary wasps to obtain a better understanding of the relationships between these insects and the environments for which each is specialized. Most of these wasps stock their nests with other arthropods, and it is on these stored materials that the young of the wasps feed. The wasps being studied have a significant effect on the arthropod fauna of the areas they inhabit.

8. Mites. The Tarsocheylidae, a new family of prostigmatic mites, has been described.

B. Identification of Insects, Mites, and Ticks.

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied in support of Federal and State research, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 32,261 lots of insect material was received for identification. Well over 284,000 specimens were examined. A total of 77,175 identifications was made and reported. Specimens were not accepted for identification unless there was a justifiable reason for the service since there is a backlog of material awaiting study.

The sources of material and the numbers of identifications made for the specimens received from each are shown in the following table:

Source	Number of Identifications	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	19,417	25.16
Plant Pest Control Division	5,168	6.70
Entomology Research Division	3,976	5.15
Forest Service	1,647	2.13
Agricultural Marketing Service	513	.67
Other Federal Agencies	2,603	3.37
States and Insular Possessions	18,377	23.81
U. S. individuals	18,961	24.57
Foreign agencies and individuals	6,513	8.44
Total Determinations	77,175	100.00

Many of the specimens received for identification are of much interest, either representing new species not previously in the National Collection, or documenting new distributional data. For these reasons, 49,309 specimens were added to the Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 2,015 publications which contained 5,177 articles of interest to insect taxonomists. Reference (by author) cards to these articles totaled 7,556. A total of 2,136 articles was catalogued in depth and from this effort 25,455 file cards were made up on which data of significance to taxonomists were recorded. The cards are of continual use in both research and service activities and the file for each specialist is kept immediately available to him.

During the year 167 visitors obtained aid on taxonomic or nomenclatural problems. The visitors remained for varying lengths of time, from an hour or so to several weeks. The significant increase in visitors over that recorded for the previous year is explained in part by the influx of zoologists to Washington to attend the XVIth International Congress of Zoology. Many of the visitors were entomologists interested in insect taxonomy.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology and Biology of Insects and Related Arthropods.

1. Leafhoppers. More than 50,000 specimens of leafhoppers have been collected in East and West Pakistan and much of the material has been mounted and thus made available for study (P. L. 480 A17-ENT-10). Well over a hundred species have been segregated in the material of the subfamily Typhlocybinae studied thus far and this number is certain to be augmented as the collections and critical study continue.

2. Beetles. Pupae of the boll weevil can be distinguished from those of the thurberia weevil by structural differences. External anatomical characteristics have been discovered in the boll weevil that can be used to separate the sexes.

3. Two-winged Flies. The family Tephritidae includes numerous species of fruit flies that are of major economic importance. In order that the species already occurring in our Western States may be quickly recognized, a comprehensive study of the fruit flies of California was published. The publication permits a ready means for separating the native fruit flies from any species that may accidentally be introduced into Western North America.

The black flies of our Northeastern States, numerous species of which attack warm-blooded animals, were monographed as part of a comprehensive study of the Diptera of Connecticut.

4. Hymenopterous Insects. Leaf-cutting ants of the genus Atta may cause significant damage to vegetation, and one species, A. texana, is an important pest of young pines in Louisiana and Texas. A key has been prepared to separate the species in Mexico and the United States. It is based on characters of the major worker ants since identifications of intermediate workers, females and males is much more difficult. Collection of major workers can be a problem, however, since they are often found only in the deep parts of the colonies.

5. Mites. Two major contributions to knowledge of the classification of spider mites treat the species of Arizona and those of Central America. Each treatment contains full illustrations of the anatomical characteristics by which the known species may be recognized.

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AREA NO. 21. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that kept them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements and the most effective manner of utilizing natural control agents, if they are to be used to maximum advantage. There is growing concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including puncture vine, Scotch broom, Dalmatian toadflax, Mediterranean sage, Russian knapweed, and halogeton. Research on insects affecting aquatic weeds, especially alligatorweed and water hyacinth, is being conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. In the United States a receiving station and laboratory is maintained at Moorestown, N. J., where major emphasis is given to receiving, propagating, and transshipping insect parasites to proper liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Albany, Calif. Studies regarding entomophagous insects are also conducted at Riverside, Calif. The work at Albany and Riverside is conducted in cooperation with the University of California and the California Experiment Stations.

The Federal scientific effort devoted to research in this area totals 15.0 man-years. Of this total, 2.5 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.0 to search for and importation of foreign insect enemies of weeds; 6.5 to basic biology, physiology, nutrition and evaluation; 3.0 to receipt, liberation and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

Twelve grants for P. L. 480 funds have been executed for projects directly concerned with the study of insect parasites and predators. Nine of these projects involve exploration for beneficial species that might be shipped to this country for trial and release against agricultural pests here. These projects are as follows: Two projects - one in India (A7-ENT-5) and one in Pakistan (A17-ENT-8) - are for a study of parasites and predators of rice insects; two in the same two countries (A7-ENT-9, India, and A17-ENT-7, Pakistan) are for studies on parasites and predators of corn borers; one in India (A7-ENT-1) is for similar studies on sugarcane borers. Two projects - one in Pakistan (A17-ENT-5) and one in Poland (E21-ENT-2) - are for studies of the biological control of scales and aphids on fruit. Two projects, A7-ENT-2 in India and S9-ENT-1 in Uruguay are for surveys of crop pests and their parasites. These 9 projects call for 40 professional man-years annually. The other three projects directly concerned with studies of insect parasites and predators are A7-ENT-8, India (mass propagation), A7-ENT-17, India (rhinoceros beetle), and S3-ENT-7, Brazil (catalogue). These three projects call for 8 professional man-years annually.

Grants for two P. L. 480 projects have been executed for studies on the biological control of weeds. Project A17-ENT-9, Pakistan, is for a general survey of insects affecting noxious weeds, and Project A7-ENT-7, India, is concerned with studies of insects affecting witch weed. These two projects require 5 professional man-years annually.

PROGRAM OF STATE EXPERIMENT STATIONS

A good research program is in progress at the State stations to identify and determine the distribution and abundance of parasitic and predatory insects that feed on agricultural insect pests and mites. In these studies biological and ecological data are gathered on these beneficial insects and their usefulness as a means of control is evaluated. Studies are made of entomophagous insects that feed on the insect pests attacking alfalfa, olive, cotton, tobacco, sweet corn, vegetable, greenhouse and other crops. A number of aphid pests have been imported without bringing their natural enemies with them. Parasite and predator introductions may establish biological control. Basic information on parasites and predators of forest insects is being obtained so that methods and techniques for biological control can be developed. The parasites of the green stink bug, which was recently found in Hawaii, are being studied.

Research is in progress on the influence of climatic factors on growth reproduction, survival and behavior of parasites and predators on selected insect pests. Climate often influences or modifies the efficacy with which natural enemies control the abundance of pests. Nutritional studies are made on parasites and predators and their host to promote more efficient mass production and utilization. Strip harvesting and other means of maintaining parasite and predator populations in nature are under investigation. Identification and classification of insect parasites and predators are being studied. By determining which insecticides may be harmless to or have the least detrimental effect on parasites and predators an integrated control program may be developed whereby both chemical and biological control are utilized to supplement each other.

A total of 12.3 man-years is devoted to this area of research by the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. Fifteen species of parasites or predators of seven different insect pests were collected in Europe for introduction into the United States, and 20 species of parasites or predators of five insect pests were sent to the United States from P. L. 480 projects in India, Pakistan, and Spain. With the exception of one species, which was sent directly to the Biological Control Institute in Belleville, Ontario, Canada, all of this material was shipped to Moorestown, N. J., for screening, testing, and transshipment to liberation points throughout the United States or to Division laboratories for further testing and propagation. Eight species were reared or field collected at Moorestown, N. J., and two at Riverside, Calif., for liberation and testing in this country. In addition one species was sent to Poland and several were sent to Iran directly from California for use against aphids and citrus pests.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Enemies of Weeds. Two insect enemies of weeds were introduced into the United States for the first time. Apion fuscirostre, a weevil which attacks the seeds of Scotch broom, was introduced from Europe, and a flea beetle, which feeds on the foliage of alligatorweed, was introduced from South America. In addition two European species, already established in this country, were collected in California for further colonization. A caterpillar which attacks tansy ragwort was recolonized at one location in California and a seed weevil, which attacks puncture vine, was widely distributed in several Western States.

C. Basic Biology, Physiology, Nutrition, and Evaluation.

1. Cereal Leaf Beetle. Several promising parasites were found that attack two species of cereal leaf beetles that were studied in Europe during the 1964 season. In southern France and in Italy south of Leghorn, Oulema melanopa was the only species of the beetle found, but north of these locations, Oulema lichensis was also present and often more abundant than melanopa. In no location were economically important host populations present. No microorganisms attacking the beetles were encountered. One of the most important parasites is an ichneumonid which overwinters in the cocoon stage. Tests are being conducted to determine the optimum method for hibernating the species.

2. Alfalfa Weevil. Studies regarding the phagycytosis of Bathyplectes curculionis in alfalfa weevil larvae indicate that through this defense mechanism a portion of the Hypera postica population in eastern States is immune to parasitization by B. curculionis. This immunity may be genetic. A striking variability is apparent in the incidence of phagycytosis in progeny of different weevils.

The parasite Tetrastichus incertus was successfully introduced into this country in 1961. By September 1963 it had spread over an area of approximately 2,000 square miles from the original release site in Oxford, Pa. The parasite usually reaches the peak of its activity after the peak of its host's abundance in the spring. Nevertheless, in late June 1964, a remarkably high rate of 98.3% and 97.6% parasitization occurred in two fields about 1.5 miles from the release area.

3. Coccinella septempunctata. The lady beetle, C. septempunctata, is an important predator on aphids in much of the Old World. Several previous, unsuccessful attempts have been made to establish it in the United States. Large numbers of eggs were obtained at Moorestown, N. J., from beetles imported from France and Italy. About 27,000 eggs were sent to Maine, where they were used on experimental plots against the potato aphid. About 27,000 eggs were sent to Idaho for tests against the pea aphid.

4. Lygus spp. Studies regarding Lygus bugs and their natural enemies were continued at Riverside, Calif. Lygus hesperus was the principal species involved, but Lygus elisus made up about 10% of most Lygus populations. The hemipterous predators of Lygus, Geocoris spp. and Nabis spp., were very abundant in August. Nabis adults were rather heavily parasitized by a tachinid. A braconid parasite, Euphorus pallipes, collected in New Jersey was successfully reared through to the cocoon stage on both species of Lygus, in California, but all of the parasites died before transforming to the adult stage. Factors causing mortality have not yet been determined.

5. Mediterranean Sage. Two phytophagous weevils, Phrydiuchus topiarius and Phrydiuchus sp., are being tested in Rome, Italy, for possible use in the biological control of Mediterranean sage (Salvia aethiops), which is a

serious introduced pest on western rangelands. Twenty-three species of plants were used in host specificity tests. The weevils fed and oviposited normally on 4 species of Salvia and on 2 closely related species in different genera, but larvae developed normally only on Salvia.

6. Thistles. Insects attacking thistles in Italy are being studied in a search for potentially valuable thistle suppressants that might be introduced into the United States. Concurrently insects affecting artichoke are also being studied, for it would be inadvisable to introduce an insect into the United States that might become a pest of artichoke, a plant that belongs to the same group as thistles that grow as weeds. At least 15 insect species have been eliminated from consideration, because they feed on both plants.

Canada has introduced a beetle, Altica carduorum, from Europe for the biological control of thistles. A small culture sent to California has been successfully propagated in the laboratory for testing and possible release.

7. Alligatorweed. Intensive laboratory and field studies have been instigated at Buenos Aires regarding the habits and host specificity of an undetermined species of thrips, which is very abundant on alligatorweed in South America. Feeding causes a marked distortion and reduction in growth of the weed. The results of detailed feeding tests using watercress and wormseed (Chenopodium) were negative, and no evidence of feeding on seven different closely associated plants was observed.

D. Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Alfalfa Weevil. The alfalfa weevil continued its rapid spread throughout the United States. European parasites collected in France, or from infestations in the United States where they have already become established, or from laboratory propagation, were released at many locations to hasten establishment and dispersion. Almost 15,000 Tetrastichus incertus were propagated. This species is now well established in Pennsylvania and New Jersey. Liberations were made in New Jersey, Kentucky, Illinois, Colorado, Idaho, California, and Washington. Small colonies of Microctonus aethiops were liberated in California, Pennsylvania, and Kentucky, and an undetermined species of Microctonus ("black") was colonized in California. Bathyplectes curculionis was obtained from California for release in Kentucky. Another very promising European species, B. anura, was released in New Jersey and Pennsylvania during 1963 and it has become well established at the release point. It has one generation annually, and the winter is spent in the cocoon stage. This past season 5,568 cocoons were collected in France and are being held for adult emergence and release in 1965. About 3,500 Dibrachoides druso were also collected in France, and good sized colonies were liberated in New York, Illinois, and Colorado. This species has not yet been recovered in this country.

2. Cereal Leaf Beetle. Two promising parasites of the cereal leaf beetle were collected in Europe. About 160 adult Tetrastichus sp., which is a gregarious larval parasite, were imported and liberated in Indiana. The second species, Tersilochus sp., is a solitary, ichneumonid larval parasite. It has only one generation a year, and overwinters in the cocoon stage. Only 26 adults were sent to this country this year, but approximately 700 overwintering cocoons were collected and are being held in hibernation. This species was very common near Rome, Italy, where it appears to be a prime factor in preventing the host from causing economic damage.

3. Pea Aphid. Aphidius smithi, an important parasite of the pea aphid, was successfully introduced into this country and established in California several years ago. This year about 8,000 were sent to Utah and 10,000 were sent to Colorado from collections made in California. A small colony of 150 adults was sent to Washington from New Jersey.

4. Brown Soft Scale. A culture of Encyrtus lecaniorum was maintained at Moorestown, N. J., following the recovery of this parasite in Israel in 1962. In the fall of 1963, 113 adults were sent to Weslaco, Tex., for propagation or release. Arrangements were also made to obtain 2 additional parasites from California. Infested host material was propagated on citron melons and shipped to Weslaco from Riverside, Calif. These shipments produced 5,615 Metaphycus stanleyi and 4,300 M. luteolus.

5. Vetch Bruchid. A total of 669 adult Dinarmus acutus (formerly Bruchobius mayri) were obtained in France for release against the vetch bruchid in Oregon.

6. Lygus Bugs. Two hundred sixty-six adult Euphorus pallipes and 13 tachinid puparia reared from Lygus bugs in New Jersey were sent to California in 1963, and 32 adults and 524 cocoons of E. pallipes were shipped in 1964. A total of 215 adult Euphorus were released near Riverside.

7. Miscellaneous Insects. Several small lots of insects were shipped for testing and release. In the spring of 1964, a shipment of 460 Lypha dubia puparia was sent directly to the Biological Control Institute in Belleville, Ontario, Canada, from France. These parasites of the European pine shoot moth were reared from collections made in Germany in 1963 and held in hibernation at the European Parasite Laboratory. Cherry fruit fly puparia collected in Switzerland in 1962 produced 122 adult Opius rhagoleticolus in July 1963. These were shipped to California from France via Moorestown, N. J. A shipment of 1,000 pupae of Cryptolaemus montrouzieri plus host material to yield Metaphycus helvolus, M. stanleyi, and M. lounsburyi was sent to Iran from California for release against citrus pests. A small shipment of citrus whiteflies was sent from Puerto Rico to the Canary Islands to produce parasites for release against the woolly whitefly. About 1,700 Lysiphlebus testaceipes were sent to Poland from California at the request of Polish entomologists.

8. Puncture Vine. The European seed weevil, Microlarinus lareynii, was colonized rather extensively in the West in the fall of 1963. About 20 colonies of 200 weevils each were liberated in 9 Western States.

9. Scotch Broom. The first releases of the European seed weevil, Apion fuscirostre, which attacks Scotch broom, were made in California in the spring of 1964. About 10,000 were released altogether in about 10 locations.

10. Alligatorweed. The first releases of the South American flea beetle, Agasicles n. sp., were made against alligatorweed in the spring of 1964. About 500 were released in Los Angeles County, California, and about 3,000 on the Savannah National Wildlife Refuge in South Carolina. The beetles were observed feeding heavily and reproducing well at the Refuge.

11. Tansy Ragwort. The cinnabar moth, Tyria jacobaeae, which was successfully established in California and Oregon against tansy ragwort has increased slowly at liberation points. It was possible to collect about 2,000 fifth instar larvae in one location in 1963, though, and these were released at another favorable release site in California.

12. Miscellaneous Insects. Twenty-nine adults and 50 larvae of Altica carduorum, the beetle imported by Canada for the control of thistles, were received at Albany, Calif., from Belleville, Ontario, for experimentation and propagation. A shipment of 185 Agrilus hyperici, which attacks klamath weed, was sent to Belleville.

13. P. L. 480 Projects. Investigations of parasites, predators, and pathogens of sugarcane borers and corn earworms are being conducted in India under P. L. 480 Projects A7-ENT-1 and A7-ENT-9. Several small shipments of parasites reared from these hosts were received at Moorestown, N. J., and transshipped to the Grain and Forage Insects Research Branch laboratories at Canal Point, Fla., and Tifton, Ga., for study, propagation, and possible release. Shipments to Florida included 66 adult tachinid flies, Sturmiopsis inferens, and the following ichneumonids: 90 Bracon chinensis, 94 Rhaconotus signipennis, and 13 Centeterus alternecoloratus. Shipments to Tifton included 92 Eriborus sp. and 8 Ecphoropsis sp., both of which are ichneumonids, and the following adult tachinids: 197 Drino sp., 14 Exorista sp., 12 Eucarcelia sp., 69 Tachinid A, and 2 Tachinid B.

Shipments of balsam woolly aphid predators were received from two Forest Service P. L. 480 Projects, A7-FS-7 India and A17-FS-5 Pakistan. The bulk of the material came from India, and most of it was sent to the Southeastern Forest Experiment Station in Asheville, N. C., as follows: 3,543 Leucopis sp. adults, 1,496 hemerobiids, mostly cocoons, and 379 Tetraphleps sp. adults. One shipment of 153 Tetraphleps sp. adults was sent to Oregon.

Gypsy moth parasites recovered in Spain on P. L. 480 Project E25-FS-10 were also handled for the Forest Service. A large number of tachinid puparia, Apanteles cocoons, and chalcid adults were received at Moorestown, N. J.,

where immature stages were reared out. All were screened and turned over to interested parties. A total of 18,492 Tricholyga segregata was obtained and released in Connecticut, Vermont, New York, and New Jersey; 15,022 Brachymeria intermedia were released in Connecticut, New York, and New Jersey; and 2,898 Apanteles porthetriae were released in Connecticut and New Jersey. None of these species is positively established in this country, although 1 adult of Brachymeria was collected about 20 years ago in Massachusetts.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAMS

Search for and Importation of Foreign Parasites and Predators of Insect Pests

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AREA NO. 22. INSECT PATHOLOGY

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria, and nematodes affecting insects. At the Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., studies are in progress on mutability-induced changes in virulence of insect diseases, and resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all sporeformers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to Division, State, and University laboratories.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements. A cooperative project to study the progress of the non-inclusion virus disease of the citrus red mite, through electron microscopy, has been set up with the Fruit and Vegetable Insects Research Branch. A second cooperative study on the serology of this virus is under way in cooperation with the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch, involving the electron microscopy of the two-spotted mite.

A second study with this Branch has been initiated to study the serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease.

The Federal scientific effort devoted to research in this area totals 9.0 professional man-years. Of this number 2.6 is devoted to virus diseases of insects; 4.5 to bacterial, protozoan, and fungus diseases of insects; 1.0 to nematodes and their associated bacteria pathogenic to insects and 0.9 to discovery and study of new pathogens.

Additional research is in progress under P.L. 480 funds at The Institute of Plant Protection, Poznan, Poland (E21-ENT-6, 1.5 man-years) and at the Annamalai University, Madras, India (A7-ENT-20, 3.0 man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

The research on the diseases of insects and methods of using these to control pests is active at the State stations. The diagnosis of insect diseases including the development and improvement of methods, techniques and processes, the systematizing, cataloguing and analyzing of symptoms, pathological changes and diagnostic procedures are being studied. The possibilities of in vitro cultivation of insect viruses by means of tissue and cell culture, the phenomena of virus infection, and the chemical and physical properties of insect viruses are being studied. Basic knowledge is being gained relating to the cause, pathogenesis, and pathology of infectious diseases of insects. The properties and characteristics of the pathogens--their virulence, infectivity, survival and dispersal capacity; the properties and characteristics of the host populations--their susceptibility, resistance and methods of transmission; the effect of environmental factors on the pathogen and host; and the factors which govern the form and shape of the epizootic curve and the initiation, development and cessation of an outbreak of disease among insects are being studied. Means of mass production and application of naturally occurring pathogens are being developed. Native and introduced pathogens including commercially produced materials are being disseminated and evaluated for their effectiveness in the field. Optimum temperature requirements for pathogens of insects are being determined. Milky disease is being studied to determine its effectiveness in the control of Japanese and oriental beetles and the physiology of its sporulation and germination. Control of insect pests of cole crops through the use and manipulation of insect pathogens is being developed.

A total of 9.9 man-years is being devoted to this area by the State stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Virus Diseases of Insects

1. A Virus-like Organism in Unhealthy Corn Borer Larvae. Recently a disease was reported by the Grain and Forage Insects Research Branch,

Ankeny, Iowa, in larvae of the European corn borer. Electron microscope examination of thin sections taken from affected larvae showed the presence of a virus-like organism of the non-inclusion body virus type. The microorganism was found exclusively in the adipose tissue of diapausing larvae. The virus-like particles are of hexagonal shape, and approximately 60 mμ in diameter. Positive identification of these particles as viruses awaits demonstration of Koch's postulates to prove their infectious capabilities. Difficulty in obtaining diseased larvae, due to adverse weather conditions has frustrated attempts to collect and purify sufficient material for these tests.

2. Citrus Red Mite Non-inclusion Virus Disease. (a) Serological Studies. The Fruit and Vegetable Insects Research Branch at Riverside, Calif., supplied virus-infected mites for virus antigen extraction and non-infected mites for controls in serological investigations. The control material was sent to Dr. J. Krywienczyk, serologist, at the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada. The infected mite material was macerated and the virus material was collected and cleaned by a system of differential centrifugation. The virus material represented 5% of the wet weight of mite tissue, a surprisingly large amount. The clean virus material was sent to Krywienczyk, who soon reported it to be a good antigen, and distinct serologically from the host tissue. Attempts to identify the virus by means of a highly sensitive fluorescent antibody technique on sectioned frozen tissue failed. Other sensitive serological methods are being investigated. (b) Histopathological Investigations. Early investigations of diseased citrus red mites by Smith, Munger, and Gilmore established that many of the virus-infected mites contained large crystals of undisclosed composition. Uninfected mites never have the crystal internally. No attempt has been made to describe to internal infectious pathology of the virus. Electron microscopical investigations are under way to elucidate the mode and site of multiplication of virus in the mite cells and to determine the nature of the crystals formed in infected individuals. (c) Nucleic Acid Studies of Arthropod Viruses. Nucleic acid analyses of two arthropod viruses, both of potential value in biological control, have been made. The first virus studied was the non-inclusion body virus of the citrus red mite. This virus proved to be an RNA virus, ribonucleic acid comprising 10.6% of the dry weight of the isolated particles. Deoxyribonucleic acid (DNA) was not found. The second virus form studied was the nuclear polyhedrosis of the corn earworm, or bollworm in cotton (Heliothis zea). In agreement with reports of similar inclusion body analysis of silkworm nuclear polyhedrosis virus, a duplexity of nucleic acid was found. Representing the H. zea virus particles included in the polyhedra, 6.81 μg of DNA/mg of polyhedral material was detected. However, 2.02 μg of RNA/mg of polyhedra was also found. While the presence and function of RNA is not known, it is incorporated with the polyhedral protein and not with the virus particle. It has been speculated that it may actually be "messenger RNA," which functioned in the synthesis of DNA in the cell nucleus, and was subsequently trapped in the polyhedral protein which crystalizes around the virus particles.

3. Mammalian Toxicology of Nuclear Polyhedral Viruses of Insects. Recently, a protocol of tests to determine the mammalian toxicology of insect nuclear polyhedral viruses was presented to and approved by the Food and Drug Administration. It includes allergenicity tests on guinea pigs, inhalation tests on mice and guinea pigs, intravenous and intracerebral injections in mice, and per os feeding of mice, using the whole polyhedra and isolated particles of insect viruses. A complete series of tests was carried out using the nuclear polyhedral virus of the corn earworm, Heliothis zea. Approximately 200 animals were included in tests and controls without a single death due to the virus. One guinea pig died shortly after exposure to inhaled polyhedra. Tissues of organs from this pig have been prepared for histological examination, and the entire inhalation test was repeated. No deaths were encountered during the second test. These results will be reported in a publication, and a request to carry out human tests has been submitted to the proper authorities.

4. Polyhedrosis of Pine Sawfly, Neodiprion pratti-pratti. Samples of purified polyhedra and of freeze-dried infected insects were furnished through the Beltsville Forest Insects Laboratory to the Virginia Department of Forestry to augment their stocks of this virus for propagation purposes. There has been no opportunity during the current year to extend our studies with this virus.

5. Granulosis Virus Disease of Red-banded Leaf Roller. Bioassay of frozen stocks of this virus showed that the material is still highly infectious after 8 years of frozen storage, and insects exposed to leaves inoculated with suspensions containing 10^6, 10^7, and 10^8 virus granules per cc were consistently infected. Microscopic examination of the material showed that the granules were present in large clumps after thawing, and hence freshly prepared virus suspensions should be employed for definitive dosage-infection tests rather than the frozen stocks. Preparation of fresh material for these tests is now under way.

6. Granulosis Virus Disease of Codling Moth Larvae. Studies in cooperation with the Yakima, Wash., laboratory of the Fruit and Vegetable Insects Research Branch showed that cultures of codling moth propagated for sterile male release were heavily infected with a granulosis virus disease. Improved methods of sanitation were recommended to prevent losses from this disease and specimens of the virus material were obtained for further study. Consistent infection of last instar codling moth larvae was obtained when healthy insects were injected with dilute blood suspensions from living diseased insects. Injected larvae held at 75° F. required somewhat more than 20 days to succumb to the disease, and most of the insects died as larvae. These were found to contain large numbers of virus granules as did also the smaller number that died as pupae. Only a small percentage of the injected larvae emerged as adults. Larvae similarly injected but held at 84° F. emerged as adults as did also larvae injected with sterile water as controls. Electron micrographs of the material injected and of larvae dying in the test

showed that the characteristic granules were present in both groups. Suspensions of the virus granules from infected insects were partially purified by passing them through Millipore filter membranes 0.22 micron pore size. Sedimentation studies with the purified suspensions showed that the granules could be sedimented quantitatively at 11,500 RPM (13,000 x gravity).

B. Bacterial Pathogens of Insects

1. Taxonomic Studies of Crystal-forming Bacterial Pathogens. Thirty-three strains, varieties, and species of crystal-forming bacteria, related to Bacillus thuringiensis, have been examined in great detail. According to a resolution passed at the International Symposium of Identification and Assay of Viruses and Bacillus thuringiensis, held on July 13, 1964, at London, England, the legitimate isolates of B. thuringiensis related crystal formers will be typed at Beltsville, at the Institute of Pasteur in France, and by J. Norris in England, and will be deposited at Beltsville as one of the three repositories in the world for crystal-forming insect pathogens.

2. Investigation of Fly Toxin from Bacillus thuringiensis. Two varieties of B. thuringiensis which produce an exotoxin, the so-called fly toxin, were extensively examined to determine the relationship existing between per os toxicity for the house fly and dipicolinic acid either as the free acid or its calcium salt. The in vitro spectrophotometric analysis of 72-hour broth culture supernatants to determine the level of toxin production compared with the amount of U. V. absorbent component (at 270 mμ) in laboratory cultures did not agree with findings from commercial preparations. The laboratory preparations examined were totally lacking in the characteristic triple peak of calcium dipicolinate or its free acid, whereas biological examination indicated a high titer of toxin. Furthermore, the U. V. analysis of the toxic solutions revealed the total absence of any characteristic spectrum in contrast to the commercial preparations. Other varieties of B. thuringiensis will be examined for spectral or chemical markers in relation to toxin production.

Initial investigations suggest that the mode of entry (action) of the B. thuringiensis toxin is by ingestion. House fly larvae readily succumbed when the toxin was injected or when allowed to freely migrate in, and feed on toxic solutions. However, no apparent toxicity was observed when the mouth parts were ligatured and the larvae exposed to toxic solutions (topical application).

3. Effect of Fly Toxin on Honey Bees. Fly toxin extracted from a commercial preparation of Bacillus thuringiensis was fed at different concentrations to larval and adult honey bees (Apis mellifera). The lowest concentrations (0.312 mg/ml of sucrose solution) killed 5.4% of the larvae after 12 days with nearly 95% emerging as adults, whereas the highest concentrations (20.0 mg/ml) killed all but 10.8%. No kill was recorded after 5 days when a concentration of 0.625 mg/ml was fed to adult bees, but the high concentration of 20 mg/ml killed nearly 100% in 4 days.

These early laboratory tests with honey bees showed the necessity for large scale tests in the field using a variety of agents. Eighteen observation hives, each with three frames and with glass fronts, were constructed inside a wooden building for these tests. Introduced colonies were allowed to build up strength before treatments were begun. The following are the treatments per hive and amounts of each of the microorganisms employed in the tests: red-banded leaf roller granulosis virus, 50 x 10^9 particles; cabbage looper polyhedra virus, 10 x 10^9 polyhedra; corn earworm polyhedra virus 10 x 10^9 polyhedra, Beauveria bassiana 10 x 10^9 spores; Bacillus thuringiensis 50 x 10^9 spores; and crude B. thuringiensis exotoxin 2000 mg.

Each treatment was duplicated with three hives as controls. Three weeks after beginning treatments, no deleterious effects were observed with one exception. The massive dose of B. thuringiensis exotoxin caused a definite weakening of the colonies. Dead larvae and pupae were noted after 10 days in both colonies and mortality approached 100% in one test colony. Observation of the colonies will be made until the spring of 1965. This work is being carried out cooperatively with the Apiculture Research Branch.

4. Effect of Fly Toxin of Lepidoptera and Diptera. Fly toxin, partially purified by passing the crude extract through Sephadex G 50 columns, was fed in 25 mg/10 ml, 50 mg/10 ml, and 100 mg/10 ml of media to third instar larvae of the Indian-meal moth and the greater wax moth. The doses used were sufficient to kill all house fly larvae fed the same concentration. All larvae of the moths survived and pupated, emerged and laid viable eggs. All pupal weights were normal.

Third instar larvae of the salt-marsh caterpillar were fed for 10 days on kale bouquets dipped in crude fly toxin preparation containing 100 mg per ml. All larvae pupated and pupal weights were normal. All pupae emerged and laid viable eggs.

In cooperation with New Zealand Government agencies, spore powder of Bacillus thuringiensis var. alesti and Bacillus thuringiensis fly toxin were sent to Dr. G. A. H. Helson for tests on the potato tuber moth. Helson found that variety alesti did not protect tubers attacked by the moth, but var. thuringiensis did protect them. Experiments to test the fly toxin, which is the suspected agent, will not be finished until Spring 1965.

Experiments using thuringiensis (fly toxin) on larvae of the blow fly (Lucilia sericata) showed a highly interesting reaction. High doses killed larvae. Low doses permitted some adult emergence but these adults were short-lived cripples, with little energy for mating and with low fecundity.

5. Mode of Action of Crystal-forming Bacteria. Previous investigators have offered the hypothesis that the intercellular cementing substances of the midgut are the sensitive site attacked by the crystal toxin from

Bacillus thuringiensis. Work at Beltsville identified this cementing substance of the greater wax moth as hyaluronic acid, a linear alternating polymer of glucuronic acid and N-acetyl hexosamine. It is reasonable to suppose that cell cementing substances in other insects are similar or related compounds. In an attempt to test this hypothesis, larval silkworms were fed cleaned crystals produced by B. thuringiensis var. thuringiensis, B. thuringiensis var. galleriae (G 2), and Bacillus thuringiensis var. sotto. The hemolymph was examined for the release of hexosamine from cementing substance in the gut when paralysis of the insect, due to crystal toxin, took place. Preliminary tests showed a progressive release of hexosamine into the hemolymph after feeding of toxin from all three varieties. In addition, the amount of hexosamine detectable at any given time varied directly with the relative toxicity of the crystals from these three varieties. Thus, in addition to providing information regarding pathological action, it is hoped that future results will permit the use of this assay as a technique for the in vitro assay of crystal toxicity.

6. Effect of High Altitude Radiation on Spores of Insect Pathogens. The research emulsion plates monitoring the spores exposed in the high altitude balloon flights (NASA 1040-N and 1042-N) have been processed and are being scanned for thin-down tracks of cosmic particles. Sample scanning of these plates shows that there is a significant increase in heavy tracks and complex cosmic stars in the flown plates as compared to those found in the laboratory and transportation controls. More than 100 hours have been devoted to systematic scanning of the plates but thus far no thin-down tracks of heavy particles have been located that would warrant investigation of their biological effect on spores. The coordinates of points of entry and exit in the emulsion of thin-down tracks of lighter particles and of points of origin of complex stars have been determined and photomicrographs of these cosmic events have been prepared for study.

C. Nematodes and Their Associated Bacteria Pathogenic to Insects

1. DD-136 Nematode for Control of Codling Moth. The field test at Kearneysville, W. Va., was continued under observation during the summer and fall of 1963 and some further evidence of the control by the nematode was obtained. Nematodes reared for a fall application in this orchard were diverted to provide sufficient material for definitive determination of the nematode sterols and the observations on the orchard were terminated.

The DD-136 nematode was applied to a test orchard at White Swan, Wash., in April to reduce overwintering codling moth larval populations and thus enhance the effect of subsequent release of sterile males. One-fourth of the trees in the orchard were left untreated to assure a carry-over of the insect population. The weather prevailing at the time of treatment was unseasonably cold and very dry and thus quite unfavorable for the application. Additional nematodes were shipped to the Yakima laboratory for a second application to open "knot holes" that harbor a large percentage of surviving overwintering codling moth larvae.

2. DD-136 Nematode Lethal to Larvae of White-Fringed Beetles and Imported Fire Ant. Tests in cooperation with the Plant Pest Control Division at Gulfport, Miss., showed that both larvae of the white-fringed beetle and the imported fire ant are quickly killed by the nematode and that the nematode develops well in these hosts. Protocols for propagating the nematode and for testing the nematode as a potential means of control of these insects in pastures where insecticide residue problems preclude satisfactory chemical treatment and for treatment of soil balls of nursery stock for certification were outlined.

3. DD-136 Nematode Attacks Mosquito Larvae. As part of a demonstration to acquaint personnel of the Corvallis, Oreg., laboratory, Insects Affecting Man and Animals Research Branch, with the capabilities of the DD-136 nematode, larvae of Aedes aegypti and of two additional mosquito species, Culex pipiens (quinquefasciatus) and Culex tarsalis were exposed on moist filter paper to infective stage larvae of the nematode. All three mosquito species were rapidly attacked by the nematode and within 15 to 70 minutes, depending on the species, invading nematode larvae could be seen in numbers within the head capsules and body cavities of the insects. Melanization of the invading nematodes was also apparent in all species.

4. Host-Parasite Relationships in Lipid and Sterol Metabolism of the DD-136 Nematode. Studies in cooperation with the Insect Physiology Pioneering Research Laboratory are under way to determine the host-parasite relationships in the nematode and other parasites, pathogens, and symbiotes of insects. Included in these studies to date are the DD-136 nematode and its associated insect pathogenic bacterium, two entomogenous fungi, Entomophthora apiculata and Entomophthora coronata, and a yeasty-like symbiote of the cigarette beetle.

The study of the DD-136 nematode has revealed the following information: (1) The nematode propagated in wax moth larvae contains two sterols in similar amounts - 55% is lathosterol (Delta 7-cholestenol) and 45% is cholesterol. The amount of total sterol in the nematode is 95 picograms (micro-micrograms) per larva. (2) The total lipid content of the nematode is about 10% of live weight. (3) The major sterol of the wax moth larvae used as hosts is cholesterol with only trace amounts of other sterols present representing carry-over of dietary sterols (Beta sitosterol, campesterol, and an unidentified sterol, possibly 7-dehydrocholesterol). (4) The total lipid content of the wax moth larvae used as host is slightly over 20% of live weight. (5) The associated bacterium does not play a part in this conversion since cadavers containing the bacterium only did not differ significantly from the insect host in either sterol content or lipid content. No lathosterol was present in such cadavers. In cadavers containing both the associated bacterium and the nematode, about 15% of the sterol present was lathosterol - about the amount expected for 33% conversion of host to parasite. (6) The lathosterol of the nematode is probably derived from the cholesterol of the host insect. Preliminary studies with female house fly adults reared on semidefined larval diets containing C^{14} cholesterol indicate this conversion.

D. General

1. Studies on Immunity in Insects. An opsonin is an antibody, found in vertebrates, which renders bacteria and other particulate matter more susceptible to the action of phagocytes. In an attempt to determine whether opsonins exist in insects, blood cells from insects immunized for 24 hours with heat killed-bacteria phagocytized injected polystyrenelatex particles (1.3μ in size) at a significantly higher rate than the controls. This suggests the presence of opsonins in insects. Although bacteria appeared to be phagocytized more often and more rapidly in simularly immunized animals, the results, using the dual living system, were irregular and definite conclusions are not possible at this stage. A large amount of this variance was due to technical difficulties. Clumping of live bacteria in tests may be a significant reaction to study further. These clumps, when phagocytized, were extremely difficult to count accurately.

Phagocytic rate is significantly depressed (approximately 75%) when serum is melanized in vitro; there is a concurrent increase in susceptibility to infected pathogens. This phagocytic depression occurred when either zymosan or tyrosinase was used as the agent causing melanin formation.

Attempts to assay insect blood for opsonins in vitro were unsuccessful. Attempts to assay for opsonins in insect blood using mammalian phagocytic cells in vitro showed promise and deserves further investigation.

Hemolymph of Galleria mellonella larvae injected with zymosan (.05 mg per 200 mg larva) demonstrated rather specific protein changes when analyzed by disc electrophoresis. The major fraction depleted by zymosan injection was shown by staining techniques to contain more lipids and by enzyme techniques more phenoloxidase activity than other protein fractions. This major fraction may be involved in the resistance of insects to pathogens since it is known that zymosan injected into insects increases their susceptibility to disease.

There apparently is no material associated with a bacterial strain highly susceptible to phagocytosis, that could be transferred to other strains that were comparatively less readily phagocytized. Since insect blood cells were extremely active in engulfing inert particles and some bacterial strains (high numbers were phagocytized in the first five minutes after injection), it is quite reasonable to propose that toxic materials causing degenerative changes and effects on insect blood cells may be part of a bacterial pathogens arsenal of protective mechanisms.

2. Effect of Hormones on Hemolymph Proteins. Recently qualitative and quantitative fluctuations in hemolymph protein have been associated with pathology caused by endocrine gland removal. All alectomized hemolymph proteins of Periplaneta americana females have been analyzed by disc electrophoresis (acrylamide gel). Qualitative differences between protein

fractions were noted. Quantitated total hemolymph protein estimates by spectrophotometric methods were made. Results indicate qualitative and quantitative changes which are being analyzed in relation to other physiological factors. This project is being conducted jointly with the Insect Physiology Laboratory.

3. Histopathology of House Flies Fed Carcinogenic Analogues of 2-Fluorenamine. Histological examination of larvae and pupae fed 2-fluorenamine derivatives revealed the following target sites---the hypodermis, fat body, imaginal nidi of the gut, and the gut epithelium.

The hypodermal cells of the larvae form melanin granules intracellularly. Later the integument adjacent to these cells becomes melanized causing large black amorphous lesions on the cuticle. Those few individuals that pupate exhibit bizarre shapes with gross malformations. These odd pupal cases are caused by hyperactive hypodermal cells which lay down an excessive amount of cuticle. In most individuals, cells of the hypodermis become separated from that layer, retain their ability to form cuticle, then form cuticular bodies in the hemocoel.

Adipose cells also form intracellular melanin granules. The pigment is then secreted, forms about each individual cell, and finally encapsulates a number of fat cells forming a large black mass in the body cavity. The cells then degenerate and become necrotic.

Cells of the regenerative nidi of the gut proliferate very rapidly, invade the gut wall and grow out into the hemocoel in long finger-like projections. It is believed that some of these cells separate from these projections and metastasize. Epithelial cells of the gut become very high and irregular, give off globules into the lumen and finally completely occlude the lumen. This result alone could account for the high mortality rate in larvae fed these vertebrate carcinogens. This work is being carried out in cooperation with the Insect Physiology Laboratory.

4. Sterol Analysis of Cigarette Beetle Symbiote. In the study of the yeast-like symbiote of the cigarette beetle, isolation was made from aseptically removed mycetomes from the adults, macerating the mycetomes in sterile water, and streaking the resulting suspensions onto slants of Hansens solution solidified by the addition of 1.5 percent agar. Representative isolated colonies were picked from the slants and transferred to peptone glucose agar. Transplants of the isolates have been sent to the Fermentations Laboratory, Northern Utilization Research and Development Division, for taxonomic study. Representative strains of these isolates (Insect Pathology Laboratory designation DD-771) were cultured in sufficient quantity for sterol analysis. These analyses indicated that the symbiote contained almost pure ergosterol.

5. Monitoring Electrophysiological Signals and Locomotor Activity of Insects. In cooperation with the Pesticide Chemicals Research Branch, the study of the electrophysiological and locomotor activity of the Madeira cockroach to detect biological rhythms, has been continued. Analysis of data digitized from the chart records of a prototype experiment designed to test the effect of a 45-minute light period followed by a 45-minute dark period to simulate the light input of a 90-minute orbitting satellite showed that there was a significantly higher level of activity during the periods of dark than during the periods of light, as might be expected with a nocturnally active insect. Frequency analysis of the data performed by Dr. Franz Halberg of the University of Minnesota Medical School indicated that the principal frequency component of activity was not represented by this 90-minute light input but that the more prominent frequency components included a 24-hour period and in the early part of the data a 4 and 8 hour component as well. Within the limits of our available equipment, a more extensive study was made, also in cooperation with Dr. Halberg, and a relatively large amount of data was accumulated on 6 additional insects under this light regimen and on 2 insects under 12 hours light and 12 hours dark for comparison. These studies confirmed the preponderance of activity during dark periods and because of the longer run of data clearly show that after the initial disturbance induced by restraint and implantation of the electrodes, all cockroaches in the tests under both lighting regimens exhibit a major 24-hour component in their rhythm. The data are not extensive enough to define whether or not the insects under the short lighting cycle show a period significantly different from 24 hours.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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AREA No. 23. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition and metabolism, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction, and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists, geneticists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect nutrition and hormones, effect of light on insect growth and development, effect of chemical carcinogens on insects; a Metabolism and Radiation Research Laboratory was established at Fargo, North Dakota, in October 1963, to conduct basic research on the metabolism of insecticides in insects and on insect sterility.

The Federal scientific effort devoted to research in this area totals 13 professional man-years. Of this number 4.0 is devoted to the biochemistry and physiology of lipids in insects, 4.0 to insect nutrition and hormones, 1.8 to effect of light on insect growth and development, 0.2 to effect of chemical carcinogens on insects, and 3.0 to metabolism of insecticides in insects and insect sterility.

Additional research in this area is provided by the following P. L. 480 projects: S5-ENT-3 Colombia (2 professional man-years); A7-ENT-6 India (2 professional man-years); A7-ENT-14 India (2 professional man-years); E21-ENT-3 Poland (1 professional man-year); and E21-ENT-4 Poland (1 professional man-year); and a research contract with the Mississippi State

University, No. 12-14-100-6895(33) (0.3 man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations have an extensive research program in insect physiology. Respiration and the intermediate metabolism of nitrogen compounds; the excretion of nitrogen in the insect; the feeding behavior and nutrition of plant sucking insects especially in aphids; the relationship between form and function in insect blood cells; the metabolism of amino acids, phospholipids and other nutrients in insects; membrane barriers in insects and the effects of temperature on them; position and chemical structure of lipid solvent extractive components of the exoskeleton of the Mormon cricket; effect of visible spectrum irradiation on growth of insects; special glands of insects that produce defense substances used for protection against predators and against systemic infection by microorganisms; location, structure and function of chemoreceptors; biochemical changes in insects as a result of development and aging; effects of X-radiation on the embryos of insects; response of insects of economic importance to radiant energy; certain Krebs cycle reactions and respiratory control in isolated flight muscle sarcosomes; major catabolic pathways for purine and pyrimidine nucleotides in flight muscles of insects; and culture methods for mandibulate and sucking plant feeding insects are being studied. Selected insects are reared on artificial diet as a means of determining their physiological and biochemical characteristics.

Mode of action of insecticides is being studied at a number of State stations. The metabolism of various insecticides in insects is studied using paper chromatography, radioactive tracers and other techniques. The effect of the size, shape and molecular configuration of insecticides and related molecules to their toxic action, the biochemical action of insecticides and drugs on enzyme systems of the insect body and the detoxification of toxic molecules in insects are being studied. Special emphasis is on organophosphorus and carbamate toxicants in much of this research. Insect resistance to insecticides is being studied with the ultimate objective of developing effective and lasting chemical control measures. The metabolic fate of certain organophosphorus insecticides after ingestion by poultry is being determined. Systematic investigations are being made of the in vitro metabolism of organophosphate insecticides by a number of tissues from several insect species. Known metabolites of insecticides are tested for their effects on the metabolic reactions of the insects. Rates and routes of entry and distribution of insecticidal chemicals in insects and intoxication, detoxification, tolerance and excretion mechanisms are being studied. The effect of population density, temperature, light and other environmental factors on toxic action of insecticides is being determined. A biological study is being made to determine the changes that occur in populations of boll weevils that influence their resistance to insecticides. Chemicals which will inhibit the detoxifying enzymes in resistant insects and etiological mechanisms which enable insects to tolerate unusual amounts of insecticides are being studied. Action of ovicides on insect and mite

eggs is being determined. Physiological effects of insecticides including synergism, antagonism and other factors are being investigated.

The effect of stresses (chemical insecticides, electric shock, thermal stress, specific enzyme inhibitors) on the elaboration of neurotoxins and on central nerve system activity are being determined. The metabolic fate of insecticides in plants is being determined. A regional project has been developed in this area. Genetic studies of the resistance of insects to insecticides are being made.

Chemical attractants, both feeding and sex stimulants, for insect pests are being studied and the use of these attractants for insect control in baits, sprays and traps is being investigated.

Chemosterilants are being studied.

There are 32.1 man-years devoted to this area at the State stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Biochemistry and Physiology of Lipids in Insects

1. Hydrocarbons of the American Cockroach. It is apparent from representative literature that there is considerable variation in the qualitative and quantitative distribution of hydrocarbons of insects. Therefore, the hydrocarbons of the American cockroach were examined as a necessary preliminary to the use of this species for a study of hydrocarbon metabolism.

A programed gas-liquid chromatogram of the total American cockroach hydrocarbons showed the presence of four major peaks which accounted for greater than 97% of the total hydrocarbon fraction. These were isolated and/or identified as n-heptacosane (11%), 3-methylpentacosane (17%) and cis,cis-6, 9-heptacosadiene (70%). Separation and identification were achieved through the use of adsorption chromatography, silver nitrate impregnated silicic acid columns, molecular sieves, gas-liquid chromatography, mass spectrometry, infrared and nuclear magnetic resonance spectroscopy, and oxidative cleavage.

The hydrocarbon fraction of the American cockroach then is the least complex of any insect yet reported and with about 97% of the hydrocarbon components identified, this insect appears to be well suited for investigations on the biochemistry of this important group of insect lipids.

2. The Utilization and Fate of β-Sitosterol in the Larva of the House Fly. Using H^3-β-sitosterol, gas-liquid chromatographic analysis and a semi-defined diet with aseptic rearing techniques, it was demonstrated that larvae of the house fly, Musca domestica, do not dealkylate β-sitosterol to form cholesterol. Furthermore, the major sterol ($>99\%$) isolated from insects reared in the above manner on a diet containing pure unlabeled β-sitosterol was identified as unchanged β-sitosterol.

β-Sitosterol, campesterol, and cholesterol were also compared in nutritional tests in which each of these three sterols served as a sole source of sterol in both the adult and larval diets. β-Sitosterol was only approximately one-fourth as effective as either cholesterol or campesterol in supporting larval growth and development. In addition, only 1.4% of the organisms from the diet containing β-sitosterol emerged as adults and these failed to produce viable eggs.

Both the biochemical data from the radiotracer studies and the biological data from the nutritional experiments conclusively demonstrated that larvae of the house fly do not convert β-sitosterol to cholesterol. Thus, pure β-sitosterol alone will not fulfill the sterol requirement of house fly larvae in entirety but serves as a "sparing sterol" which will support growth only in the presence of minute quantities of an "essential sterol."

3. Absence of Cholesterol Biosynthesis in a Primitive Insect, the Firebrat. It has been postulated that certain insects belonging to the more primitive orders may be capable of residual cholesterogenesis in contrast to the higher orders in which this capacity has been lost, through evolution. The recent report of high incorporation of dietary 1-C^{14}-acetate into cholesterol by a silverfish prompted an examination of sterol biosynthesis in the firebrat, another primitive insect. The firebrat was found to efficiently use 1-C^{14}-acetate for lipid synthesis when either fed or injected with the sterol precursor. Gas-liquid chromatographic analysis of the nonsaponifiable lipids revealed cholesterol to be the major sterol present. However, when the sterols were isolated from the nonsaponifiable lipids and the cholesterol from all the experiments combined and purified through the dibromide, the specific activity was 3.5 cpm/mg (39 cpm total). This represents about 0.001% of the radioactivity incorporated into the total lipids. The nonaseptic conditions of these experiments and the fact that a constant specific activity was not attained during the purification process both indicate that these low levels of radioactivity are insignificant. Thus, the firebrat differs considerably from the silverfish in which 10% of the total incorporated label from acetate in the lipids was in the sterol fraction. If the preliminary report on the silverfish can be confirmed, it would appear that the capacity for sterol biosynthesis, like the patterns of sterol utilization and metabolism, are characteristic for an insect species and as such are difficult to generalize.

4. Nonhomogeneity of Soybean "Gamma-Sitosterol". During the last 20 years a great number of investigators have reported on the isolation from numerous plant sources and also from a toad a sterol that exhibited a melting point of 145-147° and αD-36° to -42°. This substance was referred to as "gamma-sitosterol" and was considered to be a C-24 isomer of β-sitosterol. Two reportedly good sources of "gamma-sitosterol", soybean oil and corn oil, were examined for "gamma-sitosterol." It was shown that material exhibiting the physical properties of "gamma-sitosterol" can be isolated from these oils, but gas-liquid chromatographic analyses showed that the material consisted of a 50:50 to a 75:25 mixture of campesterol - β-sitosterol.

Since most plants contain significant amounts of campesterol and large quantities of β-sitosterol, it was concluded that "gamma-sitosterol" reportedly obtained from other sources is mot likely a campesterol - β-sitosterol mixture.

B. Insect Nutrition and Hormones

1. Effect of Allatectomy on Lipid Biosynthesis in the American Cockroach. Lipid biosynthesis was compared between normal and allatectomized female American cockroaches injected with 1-C^{14}-acetate. Allatectomized roaches had 67% more total lipids than the controls. The largest fraction by weight after column chromatography was the triglycerides, which accounted for 52% of the total weight of the lipids or 32 mg/roach in the control roaches and 68% or 70 mg/roach in the allatectomized. The phospholipid fraction (18%) accounted for less of the total lipid in the allatectomized roaches than in the controls (32%), but was about equal on a mg/roach basis.

The percent of the total injected dose incorporated into the triglyceride fraction of the allatectomized roaches was much greater than in the controls, while the percent incorporation into the hydrocarbon fraction and the phospholipid fractions did not differ greatly between the control and allatectomized roaches. This latter result confirms the existence of a large triglyceride pool in the allatectomized roaches, whereas the hydrocarbon and phospholipid pools were nearly equal in the control and experimental roaches. Graphs of the specific activity versus time showed there was no effect of allatectomy on the rate of hydrocarbon biosynthesis. The triglyceride and phospholipid fractions were definitely affected. The specific activities of these latter two fractions reached a peak later in the allatectomized roaches than in the control roaches.

The specific activity curves of fatty acid methyl esters reflected the curves of the lipid fractions. The fatty acids of the allatectomized roaches were similar both qualitatively and quantitatively to those of the controls.

The data suggest that in the American cockroach the hormones of the corpora allata regulate triglyceride and phospholipid metabolism by controlling the mechanisms responsible for their utilization. The accumulation of triglyceride in the allatectomized roaches appears to be related to the failure of ovarian development, since the ovarian lipid of the American cockroach was 70% triglyceride. Incorporation of label into the hydrocarbon fraction however did not appear to be under the control of the corpora allata.

2. Control of the Production of the Sex Attractant and the Initiation of the Regular Ovarian Developmental Cycle in the American Cockroach. Sexual maturity of the American cockroach begins with the production of a sex attractant. After mating, the sex attractant production stops and the regular ovarian developmental cycle begins. These two processes are related by common control processes and were studied together.

(a) Hormonal Control of Sex Attractant Production. Evidence has been previously given by other researchers that the corpora allata were essential to the production of the sex attractant in cockroaches. Similarly, allatectomized American cockroaches do not produce the sex attractant. Implantation of mature corpora allata or topical application of purified Cecropia extract (2 µl) or farnesyl methyl ether (4 µl) initiated sex attractant production in allatectomized cockroaches. On a quantitative basis the Cecropia extract is more potent than any of the synthetic juvenile hormone-mimicking compounds.

The average age of first production of the attractant in different groups of roaches varied from 11-16 days. Attempts to induce earlier maturation by applying Cecropia extracts or farnesyl methyl ether were unsuccessful. Implants of corpora allata were effective. However, no treated roach secreted the attractant earlier than 8 days of age. The conclusion is drawn that the hormone is effective only when the tissues are competent to biosynthesize the attractant.

(b) Cessation of the Sex Attractant. Cessation of the sex attractant production may occur naturally but it is enhanced greatly by mating. Various experimental manipulations indicate that cessation of sex attractant may also come about either by mechanical stimuli such as pinching of the bursa or by chemical stimuli transferred via the spermatophore. Mechanical stimulation generally accounts for less than 50% of the individuals losing the sex attractant. Evidence for the chemical stimuli is less direct but is inferred from experiments such as washing the bursa after removal of the spermatophore or mating with phallic glandless males. Evidence has also been obtained showing that the corpora allata are needed for maintenance of the attractant once they have initiated its production.

(c) The Initiation of the Ovarian Development Cycle. The ratio of oothecae deposited between mated and virgin roaches is nearly 7:1. This difference reflects a high activity of the gonadotropic hormone in mated roaches triggered by mating. Accelerated oothecal production (simulated mating) can be induced in virgin roaches by implanting free corpora allata into the body cavity or by severing the nerve cord connections leading to the corpora allata. These results are indicative of an inhibitory leverage placed on the corpora allata by the brain. Mating apparently frees this inhibition; whether removal of the inhibition causes the gonadotropic hormone to secrete constantly or in outbursts between oothecal formation is not known.

Various experiments and manipulations show that in the great majority of cases, regular ovarian development ensues when sperm are present in the spermatheca. No direct evidence, however, has yet been obtained showing whether sperm or seminal fluids associated with the sperm provide the effective trigger stimulus for oothecal production. Nerve cord transection immediately after mating prevents either the termination of sex attractant production or the initiation of ovarian development, and hence the brain appears to be the controlling center for both phenomena. Some evidence

indicates that impulses arising from the spermatheca travel via the ventral nerve cord to the brain. This would mean that mechanoreceptors or chemoreceptors may be present in this structure.

C. Effect of Light on Insect Growth and Development

1. Inhibition of Diapause in Pieris rapae with Photoflashes. The effectiveness of interrupting photophases as short as five minutes at 25-foot candles has been reported in tests on Pieris rapae at this laboratory. Although this is considerably shorter than has been found effective on other species, it is not the limit. When electronic photoflashes of 0.0008 second duration were applied to larvae of P. rapae four hours after the end of a 10-hour photoperiod, only 4 pupae of 105 entered diapause. Without the photoflash, 100 of 105 pupae entered diapause. The photoflash was most effective at 13 to 15 hours after onset of the light. At 20° C, 10 hours of light and a supplemental flash 4 hours later is as effective as 14 hours of continuous light, and either of these is more effective than 24 hours of continuous light.

Data now available strongly suggest that the wavelengths which are most effective for P. rapae will not interfere with red and far-red photoperiod effects in plants. The total energy employed at the wavelengths used for P. rapae is lower than that which has given responses in birds and mammals. Light from ultraviolet fluorescent lamps works for both long photoperiods and interrupting light. It should then be possible to influence diapause induction with no perceptible adverse reactions in birds or mammals.

2. Light-Dark Rhythms and Diapause Induction in Pieris rapae. Diapause incidence was measured at 18-20° C in cycles of 12, 16, 20, 23, 24, 28, 32, and 36 hours in which light comprised 0, 15, 25, 35, 45, 55, 65, 75, 85, and 100% of each cycle. Almost no diapause occurred when light was on for more than 55% of any cycle, and few insects diapaused on cycles of 12, 16, and 32 hours. The results support the hypothesis that diapause is induced by a critical dark interval in a circadian cycle that is entrained by onset of light.

D. Metabolism of Insecticides in Insects

At Fargo, North Dakota, colonies of the following insects have been started: two strains of house flies, Musca domestica, two species of grasshoppers, Melanoplus bivitatus and M. differentialis, the false wireworm, Eleodes saturalis, and a small colony of the roach Periplaneta americana.

Preliminary research has been initiated on techniques for a study on certain esterases and phosphatases and on fat body metabolism in several species of insects and on the effect of certain insecticides on these systems. Purification of some of these enzymes by chromatographic and electrophoretic procedures are underway. In an attempt to come up with a new insect control measure through the disruption of insect hormone mechanisms, work is being initiated on the effect of the corpora allata on

certain enzyme systems of the fat body.

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Kaplanis, J. N., Robbins, W. E., Vroman, H. E., and Bryce, B. M. 1963. The absence of cholesterol biosynthesis in a primitive insect--the firebrat, Thermobia domestica (Packard). Steroids 2: 547-553.
Thompson, M. J., Robbins, W. E., and Baker, G. L. 1963. The nonhomogeneity of soybean sterol--"Gamma-Sitosterol". Steroids 2: 505-512.
Baker, G. L., Vroman, H. E., and Padmore, J. 1963. Hydrocarbons of the American cockroach. 1963. Biochem. Biophys. Res. Comm. 13: 360-365.

Insect Nutrition and Hormones

Bowers, W. S. and Thompson, M. J. 1963. Juvenile hormone activity effects of isoprenoid and straight-chain alcohols on insects. Science 142 (3598): 1469-1470.

Effect of Chemical Carcinogens on Insects

Shortino, T. J., Cantwell, G. E., and Robbins, W. E. 1963. Effect of certain carcinogenic 2-fluorenamine derivatives on larvae of the house fly, Musca domestica Linnaeus. J. Insect Pathol. 5: 489-492.

AREA NO. 24. FUNDAMENTALS OF INSECT STERILITY

Problem. Basic research on insect sterility is needed to determine if this new approach can be used to control or eradicate destructive insects, thus eliminating the hazards often associated with the application of insecticides to crops and livestock or the high cost of other insect-control measures. The sterile-male technique, involving the use of gamma radiation to produce the sterility, and the release of dominant numbers of laboratory-reared sterilized males, has been utilized successfully to eliminate the screw-worm from the Southeast. The use of a sterilizing chemical in combination with a bait to attract insects already in the environment has tremendous possibilities and may prove more widely useful than radiation sterilization, because insects need not be reared in the laboratory to outnumber native insects. If a majority of native insects can be attracted and sterilized, thus outnumbering the remaining insects in the population, the same effect may be achieved without the expense of rearing, sterilizing, and releasing sterile males. This field is not necessarily limited to the use of baits containing sterilizing chemicals which insects will eat. The insects might be attracted to a light or an odor and receive a sterilizing dose of chemical through contact. Other approaches include the production of mutations in laboratory strains of insects which would not be lethal in the laboratory but would be lethal in nature. Much additional basic work is needed on the genetics and physiology of reproduction of insect pests, and on the effects of various types of sterilants, in order to determine the possibilities inherent in these new approaches to insect control and whether or not they could be utilized to destroy the many insects of economic importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term research program on insect sterility and its application to control and eradication of insect pests. Basic research on the fundamentals of insect sterility is conducted at the Metabolism and Radiation Research Laboratory established at Fargo, North Dakota, in October 1963. The research is in cooperation with Crops Research and Animal Husbandry Research Divisions and with the North Dakota Experiment Station. Research on sterility in insects produced by gamma radiation and chemosterilants directed principally toward practical application to control specific insects is also conducted at a number of field laboratories and is discussed under other areas.

The Federal scientific effort devoted to research in this area totals 4 professional man-years. Of this number 2.0 is devoted to sterility in insects by irradiation and 2.0 to sterility in insects by chemical means.

PROGRAM OF STATE EXPERIMENT STATIONS

Included in reports of other areas.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Sterility in Insects by Irradiation

Colonies of the boll weevil (Anthonomus grandis) have been established in the laboratory and a considerable research effort is currently being expanded toward the establishment of a thriving colony for use in the radiation biology studies. Investigations on this insect will be directed toward determining the basis for the unusual sensitivity to radiation for this species. Cytogenetic investigations of the reproductive organisms and the gut are already under way to determine the types of cells present as a possible basis for interpretation of radiobiological experiments.

B. Sterility in Insects by Chemical Methods

These studies will involve influence of various deleterious inherited genes under continuous introduction into laboratory populations and the cytogenetics of chemosterilant action. The insect genetics portion of this research is currently being initiated. Mutant stocks of house flies possessing genetic markers on all the chromosomes are being imported from laboratories in the United States and abroad to form a basis for additional genetic studies on the house fly.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b1(R)	Sugarbeet insect investigations			
ENT b1-1 (R)	Control methods and biological studies of insects and mites affecting sugarbeets	Mesa, Ariz.	Yes	11-G-2
		Logan, Utah	Yes	11-B-2
		Twin Falls, Idaho	Yes	11-A-2
				11-B-2
				11-C-1
		Yakima, Wash.	Yes	11-A-2
				11-C-1
				11-G-2
ENT b2(R)	Tobacco insect investigations			
ENT b2-1 (R)	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N.C.	Yes	10-A
		Florence, S.C.	Yes	10-A
		Quincy, Fla.	Yes	10-A
				10-D
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Quincy, Fla.	Yes	10-B
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S.C.	Yes	10-B
				10-C
ENT b2-4 1/ (C)	Attractants, hormones, and sterilization procedures for control of tobacco insects	Lexington, Ky.	No	
		Oxford, N.C.	Yes	10-E
		Raleigh, N.C.	No	
		Florence, S.C.	Yes	10-E
		Clemson, S.C.	No	
ENT b3(R)	Greenhouse and ornamental plant insects			
ENT b3-1 2/ (R)	Biology and methods of control of insects on greenhouse and ornamental plants	Farmingdale, N.Y.	Yes	12A-1
				12-B-1
				12-D-1
		Beltsville, Md.	Yes	12-A-1
				12-B-1
				12-D-1
				12-F-1
		Sumner, Wash.	Yes	12-A-1
				12-B-1
ENT b4	Vegetable and berry insects			
ENT b4-1 (R2)	Biology and methods of control of insects and mites affecting beans and peas	Twin Falls, Idaho	Yes	1-B-8
		Beltsville, Md.	Yes	1-B-6
				1-E-7
		Yakima, Wash.	Yes	1-F-3
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Charleston, S.C.	Yes	1-B-3
		Yakima, Wash.	Yes	1-G-3
		Mesa, Ariz.	Yes	1-C-4
ENT b4-4 (R)	Biology and methods of control of the beet leafhopper as a pest of vegetables	Twin Falls, Idaho	Yes	1-B-8
		Logan, Utah	Yes	1-B-9
ENT b4-5 (R)	Insects in relation to diseases of vegetables and berries	Mesa, Ariz.	Yes	1-H-1
		Yakima, Wash.	Yes	2-F-2
		Orono, Me.	Yes	2-F-1
ENT b4-6 (R2)	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-B-1
		Yakima, Wash.	Yes	2-A-1,2, 3,4,5
				2-B-1,2, 3,4
				2-E-1,2
ENT b4-7 (R)	Methods of preventing deleterious residues resulting from the use of insecticides on vegetables and berries	Mesa, Ariz.	Yes	1-C-4
		Yakima, Wash.	Yes	1-C-7
		Charleston, S.C.	Yes	1-A-1
				1-B-2
				1-C-2,3
		Beltsville, Md.	Yes	1-B-7
				1-C-6
		Orono, Me.	Yes	2-C-1
		Yakima, Wash.	Yes	2-C-1,2
		Charleston, S.C.	Yes	2-C-1

1/ Initiated during reporting year - supersedes in part ENT b2-1
2/ Supersedes ENT b3-2 and ENT b3-3

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-8 (R)	Investigations on the use of natural enemies and other biological methods for the control of vegetable and berry insects	Riverside, Calif.	Yes	1-D-2 1-E-4 1-A-3,4,5,6
		Beltsville, Md.	Yes	1-E-5,6,7
		Charleston, S.C.	Yes	1-D-1,2 1-E-1,2,3 1-G-2
		Yakima, Wash.	Yes	1-G-3
		Mesa, Ariz.	Yes	1-A-7
		Orono, Me.	Yes	2-D-1
ENT b4-9 (R)	Biology and methods of control of insects and mites affecting strawberries and bramble berries	Beltsville, Md.	Yes	3-B-7
ENT b4-10 (R2)(C)	Biology and methods of control of insects affecting underground portions of vegetables	Charleston, S.C.	Yes	1-A-2 1-B-1,4,5 1-E-1,2,3 1-G-2 2-B-4
		Baton Rouge, La.	Yes	1-A-2 1-B-5 1-C-2 1-G-2
		Yakima, Wash.	Yes	1-C-7 2-A-2,3
ENT b4-12 (R)	Improvement of methods and evaluation of equipment for applying insecticides to vegetable crops	Forest Grove, Oreg.	Yes	1-F-4,5
		Charleston, S.C.	Yes	1-F-2
		Yakima, Wash.	Yes	2-E-3
ENT b4-14 (R2)	Development of methods for preventing contamination of processed vegetables and berries by field insects	Beltsville, Md.	Yes	1-B-7 1-E-5,6
ENT b5(R)	Methods of treating plants and commodities regulated by plant quarantines			
ENT b5-1 (R)	Development of treatments for plants and commodities regulated by plant quarantines	Hoboken, N.J.	Yes	4-C-2 4-G-2
ENT b6	Mexican fruit fly and other fruit pests in Mexico that threaten U. S. horticulture			
ENT b6-1 (R)	Biology, ecology, and methods for control of the Mexican fruit fly and citrus blackfly	Mexico City, Mex.	Yes	4-A-2 4-E-2
ENT b6-2 (R)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	No	
ENT b6-4 (R)	Quarantine treatments for Mexican fruit fly infested fruits	Mexico City, Mex.	Yes	4-F-1 4-G-1
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1 (R)	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii	Yes	4-A-2 4-D-2
		Hilo, Hawaii	Yes	4-A-2 4-D-2
		Kahului, Hawaii*	No	
ENT b7-2 (R)	Development of new or improved mass production methods and manipulation techniques for fruit flies and their biological control agents	Honolulu, Hawaii	Yes	4-A-2
ENT b7-3 (R)	Investigation of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii	Yes	4-E-2
		Hilo, Hawaii	Yes	4-E-2 4-F-1
		Kahului, Hawaii*	No	

* Station at Kahului closed in October 1963

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Sub-heading
ENT b7-8 1/	Development of methods for eradication and control of fruit flies in Hawaii	Honolulu, Hawaii	Yes	4-E-2
		Hilo, Hawaii	Yes	4-E-2
		Guam*	Yes	4-E-2
ENT b7-9 2/	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-G-1
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R)	Studies of the codling moth and its control	Yakima, Wash.	Yes	3-A-1 3-B-1 3-E-1
		Wenatchee, Wash.	Yes	3-B-1
		Kearneysville, W. Va.	Yes	3-B-1 3-D-1
		Vincennes, Ind.	Yes	3-A-1 3-B-1 3-C-1,2
ENT b8-2 (R)	Studies of orchard mites and their control	Yakima, Wash.	Yes	3-B-2
		Wenatchee, Wash.	Yes	3-B-2
		Kearneysville, W. Va.	Yes	3-B-2
		Vincennes, Ind.	Yes	3-B-2,6 3-C-1
ENT b8-3 (R)	Studies of the plum curculio and its control	Ft. Valley, Ga.	Yes	3-B-3 3-E-2
		Vincennes, Ind.	Yes	3-B-6 3-C-2
ENT b8-4 (R)	Studies of borers attacking deciduous fruit trees and their control	Ft. Valley, Ga.	Yes	3-A-2 3-E-3
		Vincennes, Ind.	Yes	3-E-3
ENT b8-5 (R)	Studies of the pear psylla and its control	Yakima, Wash.	Yes	3-B-4
		Wenatchee, Wash.	Yes	3-B-4
ENT b8-6 (R)	Studies of miscellaneous insect and mite pests of deciduous fruits and their control	Ft. Valley, Ga.	Yes	3-B-6
		Vincennes, Ind.	Yes	3-B-6 3-C-2
		Yakima, Wash.	Yes	3-C-1
		Wooster, Ohio	Yes	3-A-2 3-B-6,7 3-C-2
		Wenatchee, Wash.	Yes	3-B-6
ENT b8-7 (R)	Investigations of nut insects and mites and their control	Albany, Ga.	Yes	3-A-3 3-B-8 3-E-4
		Shreveport, La.	Yes	3-B-8 3-F-1
		Wooster, Ohio	Yes	3-A-3 3-C-2
ENT b8-8 (R)	Grape insect investigations	Wooster, Ohio	Yes	3-C-1,2 3-F-1
ENT b9(R)	Investigations of insect and mite vectors of deciduous tree fruit viruses			
ENT b9-1 (R)	Distribution of insects and mites in and near deciduous fruit orchards infected with virus diseases	Riverside, Calif.	Yes	3-A-4
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Ft. Valley, Ga.	Yes	3-B-5
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology, and control	Riverside, Calif.	Yes	3-A-4 3-B-5
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Corvallis, Oreg.	No	
ENT b9-8	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-G-2

1/ Supersedes ENT b7-4 and ENT b7-5
2/ Supersedes ENT b7-6 and ENT b7-7
* Station at Guam closed in December 1963

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b9-9 1/	Transmission studies with possible insect and mite vectors of miscellaneous viruses causing diseases of deciduous fruits	Ft. Valley, Ga.	Yes	3-G-1
		Wenatchee, Wash.	Yes	3-G-1
		Corvallis, Oreg.	Yes	3-G-1
ENT b10	Insects of citrus and other subtropical fruits			
ENT b10-1 (R)	Biology and methods of control of citrus mites	Orlando, Fla.	Yes	4-A-1
				4-B-1
				4-E-1
		Riverside, Calif.	Yes	4-A-1
				4-B-1
ENT b10-2 (R)	Biology and methods of control of scale insects, whiteflies, and mealybugs on citrus	Orlando, Fla.	Yes	4-B-1
		Riverside, Calif.	Yes	4-A-1
				4-E-1
		Weslaco, Tex.	Yes	4-A-1
				4-C-1
ENT b10-3 (R)	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Riverside, Calif.	No	
		Honolulu, Hawaii	Yes	4-A-3
		Mexico City, Mex.	No	
ENT b10-4 (R)	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	Yes	4-H-1
ENT b10-5 (R)	Investigations of the biological control of citrus insects and mites	Orlando, Fla.	Yes	4-D-1
		Lake Alfred, Fla.	Yes	4-D-1
		Riverside, Calif.	Yes	4-D-1
		Weslaco, Tex.	Yes	4-D-1
ENT b11	Japanese beetle, European chafer, and related species			
ENT b11-1 (R)	Investigations of methods for controlling the Japanese beetle and eradicating isolated infestations	Moorestown, N.J.	Yes	12-B-2
				12-D-2
ENT b11-2 (R)	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N.J.	No	
ENT b11-3 (R)	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N.J.	No	
ENT b11-4 (R)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N.Y.	Yes	12-E
ENT b11-6	Ecology, biology, and natural control of the Japanese beetle	Moorestown, N.J.	Yes	12-C
ENT c1	Boll weevil investigations			
ENT c1-1 (R)	Biological research on the boll weevil	Florence, S.C.	Yes	9-A-1
		State College, Miss.	Yes	9-A-1
		Stoneville, Miss.	Yes	9-A-1
		Tallulah, La.	Yes	9-A-1
		Waco, Tex.	Yes	9-A-1
		Brownsville, Tex.	Yes	9-A-1
		Tucson, Ariz.	Yes	9-A-1
ENT c1-2 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the boll weevil	State College, Miss.	Yes	9-B-1
		Stoneville, Miss.	Yes	9-B-1
				9-E-1
		Tallulah, La.	Yes	9-B-1
		Waco, Tex.	Yes	9-B-1
		College Station, Tex.	Yes	9-B-1
ENT c1-3 (R)	Physiological and nutritional research on the boll weevil	State College, Miss.	Yes	9-A-1
		Stoneville, Miss.	Yes	9-A-1
		Baton Rouge, La.	Yes	9-A-1
		College Station, Tex.	Yes	9-A-1
		Florence, S.C.	Yes	9-A-1

1/ Supersedes ENT b9-7

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c1-4 1/ (C)	Discover and develop methods other than insecticidal for controlling the boll weevil	State College, Miss.	Yes	9-C-1 9-E-1 9-F-1
ENT c1-5 1/	Discover and develop methods for eradicating the boll weevil	State College, Miss.	Yes	9-D-1
ENT c2	Bollworm investigations			
ENT c2-1 (R)	Biological, physiological, and nutritional research on the bollworm and tobacco budworm	Stoneville, Miss. Brownsville, Tex. College Station, Tex.	Yes Yes Yes	9-A-3 9-A-3 9-A-3
ENT c2-2 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the bollworm and tobacco budworm	Florence, S.C. Waco, Tex. College Station, Tex. Tucson, Ariz.	Yes Yes Yes Yes	9-B-3 9-B-3 9-B-3 9-B-3
ENT c2-3 1/	Discover and develop methods other than insecticidal for controlling the bollworm and tobacco budworm	Brownsville, Tex. Waco, Tex. Tucson, Ariz.	Yes Yes Yes	9-C-2 9-D-3 9-E-2 9-F-2 9-E-2 9-F-2
ENT c2-4 1/ (C)	Bionomics of boll weevil and bollworm populations as related to cotton insect control practices	State College, Miss.	No	
ENT c3	Cotton insects other than boll weevil, bollworm and pink bollworm and insects attacking other fiber plants			
ENT c3-1 (R)	Biological, physiological, and nutritional research on miscellaneous insect and spider mite pests of cotton	Stoneville, Miss. Tucson, Ariz.	Yes Yes	9-A-3 9-A-3
ENT c3-2 1/	Development of more effective insecticides and formulations and more efficient application methods for control of miscellaneous insect and spider mite pests of cotton	Florence, S.C. Stoneville, Miss. Waco, Tex. College Station, Tex. Tucson, Ariz.	Yes Yes Yes Yes Yes	9-B-3 9-B-3 9-B-3 9-B-3 9-B-3
ENT c3-3 1/	Discover and develop methods other than insecticidal for controlling miscellaneous insect and spider mite pests of cotton	Tucson, Ariz. State College, Miss. Brownsville, Tex.	Yes Yes Yes	9-C-2 9-F-2 9-D-3
ENT c4	Pink bollworm investigations			
ENT c4-1 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the pink bollworm	Brownsville, Tex.	Yes	9-B-2
ENT c4-8 2/	Biological, physiological, and nutritional research on the pink bollworm	Brownsville, Tex.	Yes	9-A-2
ENT c4-9 3/	Discover and develop methods other than insecticidal for controlling or eradicating the pink bollworm	Brownsville, Tex.	Yes	9-D-2
ENT c5	Corn insects			
ENT c5-1 (R)	Biology and ecology of the European corn borer	Ankeny, Iowa Wooster, Ohio	Yes Yes	7-A-1 7-E-1 7-E-1
ENT c5-2 (R)	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1 7-C-1
ENT c5-3 (R)	Plant resistance to the European corn borer	Ankeny, Iowa Wooster, Ohio	Yes Yes	7-G-1 7-B-1 7-G-1
ENT c5-4 (R)	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1

1/ Initiated during reporting year
2/ Supersedes ENT c4-5 and ENT c4-6
3/ Supersedes ENT c4-2, ENT c4-3, and ENT c4-4

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c5-5 (R)	Biology, ecology, and methods of control of the corn earworm	State College, Miss.	Yes	1-B-10
				7-B-1
		Lafayette, Ind.	Yes	1-G-1
		Tifton, Ga.	Yes	1-B-10
				1-C-1
				1-F-1
				1-G-1
				7-A-1
				7-B-1
				7-C-1
				7-D-1
				7-E-1
				7-F-1
				7-G-1
ENT c5-6 (R)	Biology, ecology, and methods of controlling miscellaneous insects attacking corn	State College, Miss.	Yes	7-A-1
				7-B-1
				7-C-1
				7-E-1
				7-G-1
		Tifton, Ga.	Yes	7-B-1
				7-C-1
				7-E-1
		Brookings, S.Dak.	Yes	7-E-1
ENT c5-7 (R)	Plant resistance of corn to rice weevil attack	State College, Miss.	Yes	7-G-1
ENT c5-8 (R)(C)	Biology, ecology, and methods of control of soil insects attacking corn	Brookings, S.Dak.	Yes	7-A-1
				7-B-1
				7-E-1
				7-G-1
ENT c5-9 1/ (C)	Distribution, biology, ecology, and control of insect vectors of corn diseases	State College, Miss.	Yes	7-H-1
ENT c6	Small grain insects			
ENT c6-1 (R)	Biology, ecology, and methods of control of aphids attacking small grains	Stillwater, Okla.	Yes	7-A-2
		Brookings, S.Dak.	Yes	7-B-2
				7-F-2
				7-G-2
ENT c6-2 2/	Biology, ecology, and methods of control of mites attacking small grains	Manhattan, Kans.	Yes	7-A-2
ENT c6-3 (R)	Biology, ecology, and methods of control of Hessian fly and wheat jointworm attacking small grains	Manhattan, Kans.	Yes	7-A-2
		West Lafayette, Ind.	Yes	7-A-2
				7-G-2
ENT c6-4 (R)	Biology, ecology, and methods of control of the wheat stem sawfly	Bozeman, Mont.	Yes	7-C-2
		Minot, N.Dak.	Yes	7-G-2
ENT c6-5 (R)	Biology, ecology, and methods of control of insects attacking sorghums	Stillwater, Okla.	Yes	7-B-2
		Tifton, Ga.	Yes	7-G-2
ENT c6-6 (R)	Biology, ecology, and methods of control of soil insects and related pests of small grains	Brookings, S.Dak.	Yes	7-A-2
ENT c6-7 (R)	Distribution, biology, ecology, and control of insect and mite vectors of small grain diseases	Brookings, S.Dak.	Yes	7-H-2
		Manhattan, Kans.	Yes	7-A-2
		Baton Rouge, La.	Yes	8-B
				8-D
ENT c6-8	Biology, ecology, and methods of control of rice field insects	Baton Rouge, La.	Yes	8-A
				8-B
				8-C
ENT c6-9 (C)	Biology, ecology, and methods of control of Oulema Melanopa attacking small grains	Lafayette, Ind.	Yes	7-D-2
				7-G-2
		East Lansing, Mich.	Yes	7-B-2
ENT c7	Sugarcane insects			

1/ Initiated during reporting year
2/ Discontinued during reporting year

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Sub-heading
ENT c7-1 (R)	Biology, ecology, and methods of control of borers attacking sugarcane	Houma, La.	Yes	11-A-1 11-B-1 11-D-1 11-F-1
		Canal Point, Fla.	Yes	11-D-1
		Mayaguez, Puerto Rico	Yes	11-A-1 11-D-1 11-E-1
ENT c7-2 (R)	Biology, ecology, and control of insects other than borers attacking sugarcane	Houma, La.	Yes	11-B-1
ENT c7-3 (R)	Biology, ecology, and methods of control of insect and mite vectors of sugarcane diseases	Houma, La.	Yes	11-G-1
ENT c8	Legume and grass insects			
ENT c8-2 (R)	Biology, ecology, and methods of control of insects attacking legumes other than alfalfa and clovers	Columbia, Mo.	Yes	6-A-1 6-B-1 6-D-1 6-E-1
		Tifton, Ga.	Yes	6-A-1,2 6-B-1
ENT c8-3 (R)	Biology, ecology, and methods of control of insects attacking grasses	Lincoln, Nebr.	Yes	5-A-4 5-D-4
		Tifton, Ga.	Yes	5-A-4 5-B-4
		Forest Grove, Oreg.	Yes	5-A-4
		University Park, Pa.	Yes	5-A-4
ENT c8-4 (R)	Insect vectors of pathogenic agents affecting legumes and grasses	University Park, Pa.	Yes	5-H-1
		Columbia, Mo.	Yes	6-E-1
ENT c8-5 (R)	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-C-1,2, 4,7
		Forest Grove, Oreg.	Yes	5-C-3
		Yakima, Wash.	Yes	5-C-3
		Vincennes, Ind.	Yes	5-C-5
ENT c8-6 1/ (C)	Biology, ecology, and methods of control of aphids, leafhoppers, seed chalcids, and miscellaneous insects attacking alfalfa	Mesa, Ariz.	Yes	5-D-2 5-G-3,4
		Tucson, Ariz.	Yes	5-G-1
		Lincoln, Nebr.	Yes	5-A-2 5-G-1,2, 5
		University Park, Pa.	Yes	5-A-2 5-D-3
ENT c8-7 1/	Biology, ecology, and methods of control of insects attacking clover and sweetclover	Lincoln, Nebr.	Yes	5-A-3 5-G-8
		Forest Grove, Oreg.	Yes	5-B-3
ENT c8-8 1/	Biology, ecology, and methods of control of the alfalfa weevil	Beltsville, Md.	Yes	5-A-2 5-B-2 5-D-4 5-G-6
		Tifton, Ga.	Yes	5-A-2 5-B-2
		Tucson, Ariz.	Yes	5-A-2
		Mesa, Ariz.	Yes	5-G-7
ENT c9	General feeder insects			
ENT c9-1 (R)	Biology, ecology, and biological methods of control of armyworms and cutworms	Baton Rouge, La.	Yes	5-D-5

1/ Supersedes ENT c8-1

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c9-2	Biology, ecology, and methods of control of grasshoppers	Mesa, Ariz.	Yes	5-A-1
		Bozeman, Mont.	Yes	5-A-1 5-B-1 5-D-1 5-E-1
		Columbia, Mo.	Yes	5-D-1
ENT c9-3 (R)	Biology, ecology, and methods of control of white-fringed beetles	Florala, Ala	Yes	5-A-5 5-B-5 5-E-2 5-F-1
ENT c10	Bee culture investigations including other pollinating insects			
ENT c10-1	Biology of diseases and pests of honey bees and development of control methods	Beltsville, Md.	Yes	18-B-1, 2,3,5
		Laramie, Wyo.	Yes	18-B-3, 4,6,7
		Madison, Wis.	Yes	18-B-4, 6
ENT c10-2	Biology, breeding, and management for improvement in productivity of honey bees	Baton Rouge, La.	Yes	18-A-1, 2
		Madison, Wis.	Yes	18-A-3, 4,5,6, 7
ENT c10-3	Behavior and utilization of honey bees in the pollination of agricultural crops	Madison, Wis.	Yes	18-C
		Logan, Utah	Yes	18-C
ENT c10-4	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Tucson, Ariz.	Yes	18-D-1, 2,3
		Logan, Utah	Yes	18-D-4
ENT c10-5	Effects of pesticides and other farm practices on honey bees and other pollinating insects	Logan, Utah	Yes	18-E
		Beltsville, Md.	Yes	18-E
		Tucson, Ariz.	Yes	18-E
ENT h1	Mosquitoes, sand flies, and gnats investigations			
ENT h1-1 (R2)	Development of more effective insecticides and other materials and methods for controlling mosquitoes	Gainesville, Fla.	Yes	13-B-1 13-E-1 14-B-1 14-E-1 16-B-2 17-B-1, 2
		Corvallis, Oreg.	Yes	13-A-1 13-B-1 13-E-1 14-A-1 14-B-1 14-E-1 16-A-2 16-B-2 17-B-1
		Fresno, Calif.	Yes	13-B-1 13-D-1 14-B-1 14-D-1 16-B-2 17-B-1 17-C-1

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Sub-heading
ENT h1-4 (R2)	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting agriculture	Corvallis, Oreg.	Yes	13-A-1 13-E-1 14-A-1 14-E-1 16-A-2 16-E-2 17-A-1
		Fresno, Calif.	Yes	13-A-1 13-D-1 14-A-1 14-D-1 16-A-2 17-A-1
		Gainesville, Fla.	Yes	13-A-1 13-E-1 14-A-1 14-E-1 16-A-2 17-A-1
		Stoneville, Miss.	Yes	13-F-1 14-F-1
ENT h1-5 (R2)	Development of repellents and other materials and methods to protect man and animals from mosquitoes, sand flies, and gnats	Gainesville, Fla.	Yes	13-E-1 14-E-1 17-A-1 17-C-1 17-D-1
		Corvallis, Oreg.	Yes	13-E-1 14-E-1 17-C-1
		Kerrville, Tex.	No	
ENT h1-15 (R2)	Studies on the relationship of water and land management procedures to mosquito breeding in water impoundments and in irrigated farming areas	Fresno, Calif.	Yes	17-A-1 17-C-1
		Corvallis, Oreg.	Yes	17-A-1 17-C-1
ENT h1-16 (R2)	Studies on the biology and control of black flies, sand flies, and other gnats and their relationship to disease transmission, especially on livestock and poultry	Corvallis, Oreg.	Yes	13-A-8 14-A-8 15-A-2
		Denver, Colo.	Yes	15-E-1
		Gainesville, Fla.	Yes	17-D-3
ENT h1-17 1/ (C)	Studies on the biology and control of salt-marsh and rice-field mosquitoes in Louisiana and other Gulf Coast areas	Lake Charles, La.*	No	
ENT h2	Investigations on flies affecting man and livestock			
ENT h2-1 (R2)	Development of insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies	Kerrville, Tex.	Yes	13-A-2, 4 13-B-2, 3 13-E-2, 4 14-A-2, 4 14-B-2, 3 14-E-2, 4
		Corvallis, Oreg.	No	
		Lincoln, Nebr.	Yes	13-A-3 13-E-3 14-A-3 14-E-3
		Stoneville, Miss.	No	

1/ Initiated during the reporting year
* Station opened March 1964

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h2-1 (R2)	Development of insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies (cont.)	Beltsville, Md.	Yes	13-A-2, 3 14-A-2, 3
		Gainesville, Fla.	Yes	13-B-2 14-B-2 17-A-3 17-B-3
ENT h2-5 (R2)	Development of improved larvicides and other materials and methods for the control of screwworms and fleeceworms	Mission, Tex.	Yes	13-B-4 13-E-4 14-B-4 14-E-4 15-B-1
		Corvallis, Oreg.	No	
ENT h2-7 (R2)	Studies of irradiation and radioactive insecticides on flies and other arthropods affecting man and animals	Corvallis, Oreg.	Yes	13-B-5 14-B-5 16-B-1 17-B-2 17-D-2
		Gainesville, Fla.	Yes	13-E-6 14-E-6 17-D-3
		Mission, Tex.	Yes	13-A-5 13-E-5 14-A-5 14-E-5 15-A-1 15-D-1
		Kerrville, Tex.	No	
ENT h2-9 (R2)	Development of insecticides, attractants, and other materials and methods for the control of house flies and blow flies	Gainesville, Fla.	Yes	13-A-6 13-B-5 13-E-6 14-A-6 14-B-5 14-E-6 16-A-1 16-B-1 16-E-1 17-A-2 17-B-2 17-D-2
		Corvallis, Oreg.	Yes	13-A-6 13-B-5 14-A-6 14-B-5 16-A-1 16-B-1 16-D-1 16-E-1 17-A-2 17-B-2 17-D-2
		Lincoln, Nebr.	No	
ENT h2-11 (R2)	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Stoneville, Miss.	Yes	13-A-8 13-B-7 13-D-2 13-F-1 14-A-8 14-B-7 14-D-2 14-F-1
		Corvallis, Oreg.	No	

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h2-14 (R)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies	Gainesville, Fla.	No	
ENT h2-15 (R)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Mission, Tex.	Yes	13-A-5 14-A-5 15-A-1
ENT h2-16 (R)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Mission, Tex.	Yes	13-A-5 13-E-5 14-A-5 14-E-5 15-A-1 15-D-1
ENT h2-17	Development of physical and mechanical methods of controlling flies and other pests of livestock	Beltsville, Md.	Yes	13-E-2, 3,6 14-E-2, 3,6
ENT h3(R)	Cattle grub and bot fly investigations			
ENT h3-1 (R2)	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-7 13-B-6 14-A-7 14-B-6
		Corvallis, Oreg.	Yes	13-B-6 14-B-6
ENT h4	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-1 (R2)	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Kerrville, Tex. Stoneville, Miss.	Yes Yes	15-B-2 13-B-8 14-B-8
		Corvallis, Oreg.	Yes	13-A-9 14-A-9
ENT h4-3 (R2)	Development of improved materials and methods for the control of external parasites of poultry	Corvallis, Oreg. Kerrville, Tex. Gainesville, Fla.	Yes Yes No	16-B-3 16-B-3
ENT h4-7 (R)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Gainesville, Fla.	Yes	17-B-6
ENT h4-8 (R)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Gainesville, Fla.	Yes	17-B-6
ENT h4-9 (R)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Gainesville, Fla.	No	
ENT h4-10 (R)	Development of insecticides and other materials and methods for the control of ticks and sheep ked on animals	Kerrville, Tex.	Yes	13-A-10 13-B-9 14-A-10 14-B-9
		Corvallis, Oreg.	Yes	15-B-3
ENT h4-11	Studies on the role of lice, mites, ticks, fleas, and other arthropods in the transmission of anaplasmosis and other diseases of livestock	Kerrville, Tex. Stoneville, Miss. Corvallis, Oreg. Beltsville, Md.	Yes No No Yes	13-F-1 14-F-1 13-F-1 14-F-1
ENT h4-12 1/	Studies on the role of ticks and other arthropods in the transmission of equine piroplasmosis and on the development of insecticides and other means of controlling or eradicating vectors of the disease	Beltsville, Md. Kerrville, Tex.	Yes Yes	13-F-2 13-A-10 13-B-9
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			

1/ Initiated late in the previous reporting year

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h7-1 (R2)	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-C-2 14-C-2 15-C-2 16-C-1
ENT h7-2 (R2)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex.	Yes	13-C-1 14-C-1 15-C-1
ENT h7-4 (R2)	Development of quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT h10	Household insect investigations			
ENT h10-1 (R)	Development of measures for the control of insects in homes	Gainesville, Fla.	Yes	17-B-4, 7
		Corvallis, Oreg.	Yes	17-B-4
ENT j1	Identification and classification of insects			
ENT j1-1 (R2)	Identification and classification of hemipterous insects	Washington, D.C.	Yes	20-A-1 20-B 20-C-1
ENT j1-2 (R2)(C)	Identification and classification of beetles	Washington, D.C.	Yes	20-A-2 20-B 20-C-2
ENT j1-3 (R2)	Identification and classification of moths and butterflies	Washington, D.C.	Yes	20-A-3 20-B
ENT j1-4 (R2)	Identification and classification of grasshoppers and allied insects	Washington, D.C.	Yes	20-A-4 20-B
ENT j1-5 (R2)	Identification and classification of two-winged flies	Washington, D.C.	Yes	20-A-5 20-B 20-C-3
ENT j1-6 (R2)	Identification and classification of thrips	Washington, D.C.	Yes	20-A-6 20-B
ENT j1-7 (R2)	Identification and classification of hymenopterous insects	Washington, D.C.	Yes	20-A-7 20-B 20-C-4
ENT j1-8 (R2)	Identification and classification of mites, chiggers, and ticks	Washington, D.C.	Yes	20-A-8 20-B 20-C-5
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-6	Biological control of weeds	Albany, Calif. 1/	Yes	21-C-6 21-D-8, 9,10, 11,12
ENT j2-7 (R)	Search for and importation of foreign parasites and predators of insect pests	Riverside, Calif.	Yes	21-C-4 21-D-1, 7
		Paris, France	Yes	21-A-1 21-C-1, 3 21-D-1, 2,5,7
ENT j2-8 (R)	Search for and importation of foreign insect enemies of weeds	Rome, Italy	Yes	21-B-1 21-C-5, 6 21-D-9
		Buenos Aires, Argentina	Yes	21-B-1 21-C-7 21-D-10

1/ Transferred from Berkeley, Calif., to Albany, Calif., Sept. 6, 1963

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT j2-9* (C)	Receipt and distribution of foreign natural enemies of insect pests and weeds	Albany, Calif. 1/	Yes	21-C-6
		Moorestown, N.J.	Yes	21-C-2, 3 21-D-1, 4,6,7
		Riverside, Calif.	Yes	21-C-4 21-D-3, 6,7
ENT m1	Chemical investigations of products of natural origin for insect control			
ENT m1-14 (R)	Investigation of plants as sources of insecticides, synergists, insect repellents or attractants, or insect antimetabolites	Beltsville, Md.	Yes	19-A-2
		State College, Miss.	Yes	19-A-2
ENT m1-15	Investigation of substances naturally occurring in insects that might be used to upset their development or reproduction or otherwise affect their vital processes	Beltsville, Md.	Yes	19-A-1
ENT m2	Chemical investigations to develop synthetic organic materials for insect control			
ENT m2-1 (R2)	Preparation of synthetic organic compounds for testing as insecticides and synergists	Beltsville, Md.	Yes	19-B-2
ENT m2-4 (R2)	Development of chemical formulations for insect control	Beltsville, Md.	Yes	19-B-3
		Gainesville, Fla.	Yes	19-B-3
ENT m2-13 (R)	Chemical investigations of radioactively labeled insecticides	Beltsville, Md.	Yes	19-B-1
ENT m2-15	Preparation of synthetic organic compounds for testing as insect control or eradication agents through effects other than death	Beltsville, Md.	Yes	19-B-2
		Kerrville, Tex.	Yes	19-B-2
		State College, Miss.	Yes	19-B-2
ENT m2-16 2/	Preparation of compounds for testing as insect chemosterilants	Beltsville, Md.	Yes	19-B-1
ENT m2-17 2/	Development of basic chemical information on insect chemosterilants	Beltsville, Md.	Yes	19-B-1
		Gainesville, Fla.	Yes	19-B-1
		State College, Miss.	Yes	19-B-1
ENT m3	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-5 (R2)	Analysis of insect control chemicals, their formulations, and accessory materials	Beltsville, Md.	Yes	19-C
ENT m3-6	Determination of residues of insect control chemicals in plant and animal products and in soils	Beltsville, Md.	Yes	1-C-2, 3,6 2-C-1 3-C-1,2
		Yakima, Wash.	Yes	1-C-4, 5,7 2-C-1,2 3-C-1 5-C-1, 3,6 11-C-1
		Tifton, Ga.	Yes	1-C-1 5-C-1, 2,4,7 7-C-1
		Vincennes, Ind.**	Yes	3-C-1,2 5-C-1,5 7-C-1,2 12-B-2

1/ Transferred from Berkeley, Calif., to Albany, Calif., Sept. 6, 1963
2/ Initiated during reporting year
* Supersedes ENT j2-4
** Pesticide Chemicals Research Laboratory closed June 1964

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT m3-6	Determination of residues of insect control chemicals in plant and animal products and in soils (cont.)	Kerrville, Tex.	Yes	13-C-1 14-C-1 15-C-1
		Hoboken, N.J.	Yes	4-G-2
		Mexico City, Mex.	Yes	4-G-1
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			
ENT m4-1 (R2)	Development of formulas and dispensing equipment for aerosols to control insects	Beltsville, Md.	Yes	19-D
ENT m9	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R2)	Comparison of the toxic, attractant, arrestant, and repellent action of chemical materials to test insects	Beltsville, Md.	Yes	19-E-1
		Brownsville, Tex.	Yes	19-E-1
ENT m9-3 (R2)	Comparison of insecticidal materials in gas-propelled aerosols and space sprays	Beltsville, Md.	Yes	19-E-3
ENT m9-4	Biological evaluation of materials for insect control through effects other than death	Beltsville, Md.	Yes	19-E-2
		Brownsville, Tex.	Yes	19-E-2
ENT m10	Methods for disinsectization of aircraft (Not divided into line projects)	Beltsville, Md.	Yes	19-F
ENT m11	Development of methods of analysis for pesticides and pesticide residues			
ENT m11-2 (R)	Development of methods of analysis for pesticides and pesticide residues	Beltsville, Md.	Yes	19-C
		Orlando, Fla.	Yes	19-C
		Tifton, Ga.	Yes	19-C
		Vincennes, Ind.*	Yes	19-C
		Kerrville, Tex.	Yes	19-C
		Yakima, Wash.	Yes	19-C
ENT P 1	Insect Pathology Laboratory	Beltsville, Md.	Yes	22-A-1, 2, 3, 4, 5, 6 22-B-1, 2, 3, 4, 5, 6 22-C-1, 2, 3, 4
ENT P 2	Insect Physiology Laboratory	Beltsville, Md.	Yes	23-A-1, 2, 3, 4 23-B-1, 2 23-C-1, 2 23-D
ENT q1 1/	Insect metabolism and physiology	Fargo, N.D.	Yes	23-D
ENT q2 1/	Radiation biology and insect genetics	Fargo, N.D.	Yes	24-A 24-B
ENT-0-0-1 1/ (AID)	Studies on the biology and control of tsetse flies in Africa	Salisbury, Southern Rhodesia	No	
	P. L. 480 Projects			
A7-ENT-1	Investigations of parasites, predators, and pathogens of sugarcane borers in India	India	Yes	11-D-1 21-D-13
A7-ENT-2	Survey of beneficial parasites and predators of agricultural and horticultural crops in the Indian Union	India	No	
A7-ENT-5	Investigations of parasites, predators, and pathogens of insect pests of paddy (rice)	India	No	

1/ Initiated during reporting year
* Pesticide Chemicals Research Branch Laboratory closed June 1964

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
A7-ENT-6	Nutritional studies on the silkworm Bombyx mori L. - its requirements for vitamins and amino acids and its nutrition in relation to the mineral nutrition of its host plant, mulberry (Morus indica) and studies on the host specificity of the silkworm Bombyx mori L.	India	No	
A7-ENT-7	Survey for natural enemies of witchweed, and of water hyacinth and other weeds affecting waterways, in India	India	No	
A7-ENT-8	Developing methods for large-scale rearing of parasites under laboratory conditions	India	No	
A7-ENT-9	Investigations of parasites, predators, and pathogens of the European corn borer and Heliothis spp. in India	India	Yes	7-D-1 21-D-13
A7-ENT-10	Acarine disease problem of honey bees	India	Yes	18-B-2
A7-ENT-14	Studies on the free amino acids of insect haemolymph and the accumulation of citric acids in insect tissue	India	No	
A7-ENT-17	Control of the coconut rhinoceros beetle Oryctes rhinoceros L.	India	No	
A7-ENT-20	Studies on microbiology and pathology of insect pests of crop plants	India	No	
A7-ENT-24	Systematic and biological studies of Indian thrips	India	No	
A7-ENT-25	Research on insect pests of maize with special reference to stalk borers	India	No	
A7-ENT-26	Biology of gall midges affecting mangoes with special reference to the extent of damage	India	No	
A7-ENT-28	Taxonomic studies of several families of Mallophaga (chewing lice)	India	No	
A7-ENT-29	A study of the taxonomy of adult and larval Bruchidae	India	No	
A10-ENT-5	Host plant-vector and host plant-virus relationships of the rough dwarf virus of corn and methods for control of the disease	Israel	Yes	7-H-1
A13-ENT-3	Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung	Korea	No	
A17-ENT-5	Studies on indigenous natural enemies of scale insects and fruit flies	Pakistan	No	
A17-ENT-7	Investigations on the natural enemies of corn borers	Pakistan	No	
A17-ENT-8	Studies on the natural enemies of insect pests of rice	Pakistan	No	
A17-ENT-9	Studies on the insect enemies of noxious weeds in Pakistan	Pakistan	No	
A17-ENT-10	Studies on oriental leafhoppers (Typhlocybinae)	Pakistan	Yes	20-C-1
E8-ENT-1	An investigation of the population dynamics of Calligypona pellucida (F.) and of the nature of the injury to oats and spring wheat caused by this plant hopper	Finland	Yes	7-H-1
E11-ENT-1	Control of the olive fly, (Dacus oleae (Gmelin)) with radiation or chemical sterilization procedures	Greece	No	
E15-ENT-1	A study of Acarine disease of honey bees	Italy	Yes	18-B-2
E21-ENT-2	Studies on the possibility of biological control of aphids and scale insects and the effect of pesticides on the natural enemies of these pests	Poland	Yes	3-D-2 21
E21-ENT-3	The influence of some vitamins on the physiology of the Colorado potato beetle (Leptinotarsa decemlineata Say)	Poland	No	
E21-ENT-4	The causes and the role of diapause of insect pests	Poland	No	
E21-ENT-5	Studies on the differences in susceptibility of spider mites to acaricides and on cholinesterases in spider mites as influenced by acaricides	Poland	Yes	3-B-9

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E21-ENT-6	The nature of infectious processes caused by protozoa in insects	Poland	No	
E21-ENT-7	The development, maturation, and production of drones and the natural mating of virgin and drone honey bees	Poland	Yes	18-A-2
E21-ENT-8	Mite fauna of orchards with special reference to the relation between phytophagous and predaceous species	Poland	No	
E21-ENT-9	Insect vectors of virus diseases of various forage legumes	Poland	Yes	5-G-2 5-H-2
F4-ENT-2	Survey of the insect fauna of Egypt	Egypt	No	
F4-ENT-3	Induced sterility in males of the Mediterranean fruit fly, Ceratitis capitata, as a means of controlling and possibly eradicating that pest	Egypt	Yes	4-E-1
F4-ENT-4	Biology, ecology and utilization of insects other than honey bees in the pollination of agricultural crops	Egypt	No	
S3-ENT-1	Biology and breeding of honey bees	Brazil	Yes	18-A-2
S3-ENT-7	Catalogue of insects living on plants in Brazil and of the parasites and predators of the insects	Brazil	No	
S5-ENT-2	A biochemical study of Drosophila (vinegar flies) classification	Colombia	No	
S5-ENT-3	The metabolism of temperature acclimated Drosophila	Colombia	No	
S9-ENT-1	Studies of the parasites and predators of several insects of economic importance	Uruguay	No	
S9-ENT-3	Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay	Uruguay	Yes	17-A-4
S9-ENT-6	Systematic collections, identification, and classification of the grasshoppers of Uruguay and neighboring territories of Southern Brazil, Southern Paraguay, and adjacent provinces of Argentina	Uruguay	No	